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Oxygen and Carbon Dioxide Tensions of Arterial Blood during Heavy and Exhaustive Exercise.

By

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Arterial blood gas tensions during exercise have been studied extensively using direct measurements. The oxygen tension of arterial blood Pa_{O_2}, has been found to increase (BARTELS *et al.* 1955, ASMUSSEN and NIELSEN 1957) or decrease (LILIENTHAL *et al.* 1946, SUSKIND, BRUCE and MCDOWELL 1950, FILLEY, GREGOIRE and WIGHT 1954, LINDERHOLM 1959) during moderate to heavy exercise. Direct measurements of arterial carbon dioxide tension, Pa_{CO_2}, have shown that during exercise Pa_{CO_2} may increase above normal resting values (ENGHOFF 1938, HICKAM *et al.* 1951) or fluctuate within the normal range of variation (LILIENTHAL *et al.* 1946, SUSKIND *et al.* 1950, FILLEY *et al.* 1954, BARTELS *et al.* 1955, ASMUSSEN and NIELSEN 1956, LINDERHOLM 1959). Also indirect methods have been applied, particularly in order to estimate carbon dioxide tension of arterial blood from analysis of end-tidal air. These studies have shown similar results. During exhaustive work, however, several investigations report a decrease in alveolar CO_2 tension and hyperventilation (TAYLOR 1941,

Table I.

Anthropometric data.

Case no.	Age years	Height cm	Weight kg	THb g	Heart vol. ml	Work cap. kgm/min
1	18	185	65	830	870	1,400
2	18	171	73	875	1,140	1,800
3	17	188	74	970	1,000	1,600
4	17	180	68	720	865	1,500
5	17	175	58	630	885	1,400
6	19	171	57	695	980	1,100
7	18	177	65	695	1,100	1,300
8	17	175	71	870	945	1,300
9	17	174	67	955	935	1,500
10	17	178	69	890	1,110	1,600
11	19	171	59	640	935	900
12	19	175	73	880	1,290	1,700
13	19	180	65	700	845	1,200
14	17	182	70	800	1,160	1,500
$\bar{x}$	17.8	177.4	66.7	796	1,002	1,414

$\bar{x}$ arithmetic mean
THb = total amount of hemoglobin.

COMROE 1944, with references) but an increase has also been found (BANNISTER, CUNNINGHAM and DOUGLAS 1954).

The differing results obtained may to a great extent be attributed to the fact that the examinations were made at different work loads in relation to the working capacity. This report deals with measurements of Pa_{O_2} and Pa_{CO_2} during exercise of defined absolute and relative intensity in young, well-trained subjects. Ventilation measurements were not made in order not to disturb the normal breathing pattern and to avoid an increase in air-way resistance.

Material.

Fourteen "junior" athletes (bicycle racers) were examined. The antropometric data are presented in Table 1. The investigation was combined with a general medical examination, ECG taken at rest and in connection with a work test, roentgen examination of the heart and the lungs, examinations of blood (sedimentation rate, Hb concentration) and urine (protein, glucose). None of the subjects showed signs of disease.

All subjects, except no. 6 and 11, had a high physical working ca-

pacity, which in all cases was normal in relation to total hemoglobin (THb) and heart volume (SJÖSTRAND 1953, HOLMGREN *et al.* 1957, with ref.). The total amount of hemoglobin per kg body weight (11.95 g/kg) was not as high as in earlier examined groups of athlete cyclists (HOLMGREN et al. 1957) but the lactate concentration during heavy exercise was low (see below), indicating that they were well trained (HOLMGREN and STRÖM 1959).

Methods.

Experimental procedure: The physical working capacity of each subject was assessed according to SJÖSTRAND (1947) and WAHLUND (1948) on an electrically braked bicycle ergometer (HOLMGREN and MATTSSON 1954). ECG was recorded at rest, during and after the work test (SJÖSTRAND 1951). THb was determined by the alveolar CO method (SJÖSTRAND 1948) and the heart volume in the prone position was obtained from two-plane roentgenograms (LARSSON and KJELLBERG 1948, KJELLBERG, LÖNNROTH and RUDHE 1951).

On a following day a polyethylene catheter was introduced into the brachial artery in local anaesthesia (BERNEUS *et al.* 1954). This method permits almost free movements of the arm and makes drawing of blood samples easy even during exhaustive work. Soon after the arterial puncture an arterial blood sample was drawn from the brachial artery with the subject resting in the supine position.

The subject started to work on the bicycle ergometer in the sitting positions at a load of 600 kgm/min. The load was then increased by 300 kgm/min every 6th—7th minute until a pulse frequency of 150—184 (mean 168) beats/min was reached and was then continued for 15 minutes. At the end of this period another blood sample was drawn and the load was increased to 2,150 kgm/min. The subject was asked to continue as long as possible, usually for 1—3 minutes. During the last minute of this exhaustive work a third blood sample was taken. At the end of the work the subjects were unable to keep a constant pedalling rate and showed signs of exhaustion. The skin became cold and pale, particularly round the mouth, and cyanosis often appeared.

Blood samples were drawn in 30 ml all-glass syringes, with the dead space of about 0.2 ml filled with a 1 % heparin solution. Thirty ml of blood was taken for each analysis and the syringes were stored in a refrigerator (+ 4° C).

Analytical methods: pH measurements on whole blood were made at 37° C using a glass electrode and a potentiometric pH-meter (Radiometer PHM 3). Phosphate buffers were used as standards. The standard error of a measurement as estimated from 42 duplicate determinations was 0.002 pH units.

Carbon dioxide tension of arterial blood, Pa_{CO_2}, *and standard bicarbonate* were determined according to the principles outlined by ASTRUP (1956) using the linear relationship between pH and log P_{CO_2} of blood (see LINDERHOLM 1957, LINDERHOLM and NORLANDER 1958).

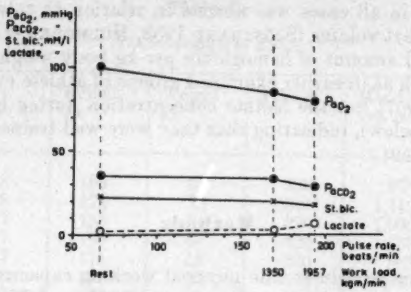

Fig. 1. Mean values of Pa_{O_2}, mm Hg, Pa_{CO_2}, mm Hg, standard bicarbonate, mM/l, and lactate concentration, mM/l (ordinate) in relation to pulse frequency, beats per minute, and work load, kgm/min (abscissa), at rest, during heavy and exhaustive work.

Oxygen tension of arterial blood, Pa_{CO_2}, was determined by the potentiometric mercury drop electrode (BARTELS 1951, BARTELS and LAUÉ 1951) within 2—3 hours after the withdrawal of the blood samples. All measurements on blood samples were made in duplicate and the measurements on blood from one syringe were completed within 1 hour. The standard curve was obtained from measurements on the same blood equilibrated for 20 min in tonometers (LAUÉ 1951) at 37° C with at least 2 gas mixtures of different O_2 tensions between 50 and 350 mm Hg and with a CO_2 concentration of about 5 per cent. There were small and insignificant differences between the standard curves of blood taken at rest and those of blood obtained during exercise, which is in agreement with earlier findings (BARTELS *et al.* 1955). The error of the method was estimated to be $\pm$ 2—3 per cent (coefficient of variations) in the range of the present measurements (cf. GORDH, LINDERHOLM and NORLANDER 1958).

Lactate concentration was determined according to the method of BARKER and SUMMERSON (1941) as modified by STRÖM (1949), cf. also HOLMGREN (1956).

Results.

The results are summarized in Tables II, III and IV and in Fig. 1.

Intensity of work.

The heavy work was performed in a relative steady state of pulse frequency and with a mean pulse rate after 15 minutes work of 168 (range 157—184) beats/min at a mean work load of 1,350

Table II.

Pulse rate and results from analysis of arterial blood taken at rest.

Case no.	Pulse rate beats per min	Lactate conc. mM/l	Hb conc. g/100 ml	pH	St. bic. mM/l	Pa_{CO_2} mm Hg	Pa_{O_2} mm Hg
1	50	1.1	14.0	7.415	23	35	115
2	66	2.5	16.2	7.425	23	33	112
3	60	1.7	13.0	7.404	23	38	—
4	60	—	13.6	7.407	21	34	115
5	68	—	12.7	7.387	20	37	103
6	60	—	14.4	7.418	22	33	103
7	70	—	14.1	7.398	23	36	102
8	76	1.4	13.9	—	—	—	90
9	64	1.3	13.8	—	—	—	82
10	58	1.3	14.3	—	—	—	81
11	84	1.1	13.8	—	—	—	78
12	66	1.8	14.2	—	—	—	103
13	76	1.8	15.1	—	—	—	120
14	64	—	15.0	7.396	21	34	108
$\bar{x}$	66.4	1.59	14.20	7.406	22.0	35.0	100.9
n	14	9	14	8	8	8	13
S. D.	8.5	0.46	0.88	0.013	1.19	1.85	14

$\bar{x}$ = arithmetic mean.
n = number of subjects examined.
S. D. = standard deviation.
Pa_{O_2}, Pa_{CO_2} partial pressure of O_2 and CO_2 respectively in arterial blood.
Stand. bic. = standard bicarbonate, *i. e.* the bicarbonate content of plasma of blood at 37° C, saturated with oxygen, and at a CO_2 partial pressure of 40 mm Hg.

kgm/min (range 900—1,500). The increase in pulse frequency between the 2nd and 15th minutes of work on this heavy load was 11.5 (range 2—21) beats/min. The mean lactate concentration was moderately increased, 2.5 (range 1.3—4.1) mM/l, and the standard bicarbonate correspondingly decreased, 2.2 (range 0—3) mM/l (Tables II and III), as compared to the values at rest. The latter were in the normal range except in case no. 2 who had a lactate concentration of 2.5 mM/l, probably due to physical exercise before the investigation. In three cases, in which simultaneous measurements of lactate and bicarbonate concentration were performed a similar agreement between the values was obtained. The Hb concentration increased by about 1 g/100 ml as compared with the value at rest.

The exhaustive work was a non-steady state work and the pulse frequency at the point when the subjects broke the work was between 176 and 205 (mean 192.5) beats per min after 2 (range 1—

Table III.

Work load, pulse rate and results from analysis of arterial blood taken after 15 min of heavy work in relative steady state, and during exhaustive work.

Case no.	Load kgm/min	Pulse rate beats per min	Lactate conc. mM/l	Hb conc. g/100 ml	pH	St. bic. mM/l	Pa_{CO_2} mm Hg	Pa_{O_2} mm Hg
Heavy work								
1	1,500	184	2.2	15.4	7.396	21	33	87
2	1,500	150	4.0	16.6	7.358	19	34	90
3	1,500	170	2.2	14.3	7.348	19	36	91
4	1,500	172	—	14.5	7.331	19	35	94
5	1,200	160	—	13.7	7.396	20	32	88
6	1,200	178	—	15.3	7.331	19	36	82
7	1,200	157	—	15.0	7.409	21	31	87
8	1,200	165	1.9	14.8	—	—	—	86
9	1,500	170	1.6	15.3	—	—	—	59
10	1,500	172	2.2	15.4	—	—	—	82
11	900	168	1.3	14.4	—	—	—	72
12	1,500	164	2.7	15.2	—	—	—	88
13	1,500	178	3.8	15.9	—	—	—	94
14	1,200	165	—	16.0	7.360	20	35	90
$\bar{x}$	1,350	168.1	2.48	15.18	7.366	19.8	34.0	84.9
n	14	14	9	14	8	8	8	14
S. D.	195	8.9	0.933	0.76	0.031	0.80	1.85	9.4
Exhaustive work								
1	2,100	205	7.5	15.7	7.353	17	29	72
2	2,100	176	8.9	16.5	7.309	17	31	92
3	2,100	198	4.6	14.5	7.510	18	16	85
4	2,100	187	—	14.9	7.314	16	29	93
5	1,800	195	—	—	—	—	—	—
6	1,800	200	—	15.5	7.271	16	36	83
7	1,800	194	—	15.5	7.387	19	30	83
8	2,000	—	6.4	15.1	—	—	—	82
9	2,000	191	3.4	15.6	—	—	—	57
10	2,000	—	4.9	15.4	—	—	—	81
11	1,500	189	3.0	14.5	—	—	—	64
12	2,000	—	7.5	15.4	—	—	—	80
13	2,000	—	4.1	16.3	—	—	—	80
14	2,100	190	—	16.0	7.288	18	40	72
$\bar{x}$	1,957	192.5	5.64	15.50	7.347	17.3	30.1	78.8
n	14	10	9	13	7	7	7	13
S. D.	174	8.0	2.06	0.60	0.082	1.11	7.47	10.2

Symbols as in Table II.

3.3) minutes work. The lactate concentration increased during this short period of work to a mean of 5.6 mM/l (range 3.1—8.9) and the mean standard bicarbonate decreased correspondingly, *i. e.* by 4.7 mM/l (from 22.0 to 17.3 mM/l). The mean hemoglobin concentration increased further about 0.3 g/100 ml.

Table IV.

Differences between data obtained at rest and during work.

Experimental conditions		Pa_{O_2} mm Hg	Pa_{CO_2} mm Hg	St. bic. mM/l
Rest — heavy work	n	13	8	8
	$\bar{D}$	16.5	1.0	2.25
	P	0.001	0.1	0.01
Heavy work — exhaustive work	n	13	7	7
	$\bar{D}$	6.7	4.1	2.43
	P	0.01	0.1	0.001
Rest — exhaustive work	n	11	7	7
	$\bar{D}$	24.6	4.9	5.0
	P	0.001	0.05	0.001

n = number of differences.
$\bar{D}$ = mean difference.
P = probability that the difference is caused by random factors.

Gas tensions of arterial blood.

At rest Pa_{O_2} was determined in 13 of the 14 subjects and varied between 78 and 120 mm Hg (mean 100.9 mm Hg); Pa_{CO_2} was determined in 8 of 14 subjects and varied between 33 and 38 mm Hg (mean 35.0 mm Hg).

During heavy work the mean Pa_{O_2} decreased significantly as compared with the values at rest to 84.9 (range 59—94) mm Hg (cf. Tables III and IV). At the same time the mean Pa_{CO_2} was slightly lower in eight cases, 34 (range 31—36) mm Hg. The difference as compared with the value at rest was, however, not statistically significant (Table IV).

During exhaustive work the mean Pa_{O_2} decreased further to a value of 78.8 (range 57—93) mm Hg. The difference between the value obtained during heavy work and that during exhaustive work was statistically significant. The mean Pa_{CO_2} in 7 cases decreased to 30.1 (range 16—36) mm Hg. The difference between the mean value at rest and that obtained during exhaustive work was probably significant (see Table IV).

The *mean barometric pressure* during the investigation was 759 (range 751—768) mm Hg.

Discussion.

As the physical working capacity is usually limited by circulation, and the pulse frequency increases almost linearly with increasing work load, the pulse rate in relation to the maximal pulse frequency (190—200 beats/min in well trained young adults (ROBINSON 1938, ÅSTRAND 1952)) may be used as an approximate measure of the relative intensity of work. The relative intensity of work can also be expressed as the observed oxygen uptake in relation to maximal oxygen uptake capacity (ÅSTRAND and RYHMING 1954).

The present results show that arterial blood-gas tensions vary with the relative work load. Consequently, measurements during exercise should be related to the relative intensity of the work in order to make results from different investigations comparable.

In earlier investigations the experimental data presented usually do not allow an estimation of the relative work load, which probably varied considerably, a fact that may partly explain the divergent results.

The mean Pa_{O_2} at rest in the present investigation, 101 mm Hg, is rather high when compared with the mean values in some earlier investigations (89—101 mm Hg, cf. LILIENTHAL *et al.* 1946, SUSKIND *et al.* 1950, BARTELS *et al.* 1955 and LINDERHOLM 1959) which all were performed at sea level. The blood samples were drawn soon after the arterial puncture and it was not attempted to obtain basal conditions. Hyperventilation might therefore have been present, as is also suggested by the low value for resting Pa_{CO_2}.

During heavy work the values for Pa_{O_2} (85 mm Hg) agrees with those found by ASMUSSEN *et al.* (1957) but were slightly lower than the mean values (88—103 mm Hg) reported by LILIENTHAL *et al.* (1946), SUSKIND *et al.* (1950), RILEY *et al.* (1954) and BARTELS (1955). Only FILLEY *et al.* (1954) at 1,600 feet above sea level found a lower mean value (82 mm Hg). BARTELS *et al.* (1955) found a slightly higher Pa_{O_2} during work than at rest, but evidently the relative work load was low.

Only few Pa_{O_2} measurements have been reported from exhaustive work. In one case FILLEY *et al.* (1954) found a Pa_{O_2} of 89 mm Hg. The mean Pa_{O_2} observed in the present investigation is significantly lower than that during heavy exercise, see Table IV.

The tendency to a decrease in Pa_{CO_2} with increasing intensity of work as observed in the present investigation agrees with some earlier observations using direct measurements on arterial blood (RILEY *et al.* 1954, FILLEY *et al.* 1954). However, an increase in Pa_{CO_2} (ENGHOFF 1938, SUSKIND 1950) or small variations (LINDERHOLM 1959) have also been reported.

During exhaustive work most earlier estimations of arterial blood CO_2 tension were made from measurements on end-tidal air and have shown both low (COMROE 1944, with references) and high (BANNISTER *et al.* 1954) CO_2 tensions. The end-tidal air, however, seems to have higher CO_2 tension during heavy work than arterial blood (ASMUSSEN and NIELSEN 1956).

It is possible that resistance to breathing through mouth pieces, respiratory valves etc. may decrease the ventilation during heavy and exhaustive work (cf. ZECKMAN, HALL and HULL 1957) and explain some of the divergent results. In this investigation there were no such hindrances to breathing. It therefore seems that in young well-trained men a decrease of Pa_{CO_2} and hyperventilation is usually present during heavy and exhaustive work.

It is remarkable that Pa_{CO_2} may reach quite low values during exhaustive work in some cases (16 mm Hg in case 3). Such low Pa_{CO_2} values do not seem to be in accordance with the idea that CO_2 is the main stimulus for ventilation during heavy and exhaustive work.

Conclusions regarding respiratory gas exchange during heavy and exhaustive work.

The ability to perform work in a steady state is mainly dependent on the O_2 transport capacity of the body and it is generally assumed that this is normally limited by circulation (HILL, LONG and LUPTON 1925, HERBST 1928) and not by lung function. If O_2 and CO_2 exchange by the lungs between the ambient air and blood were insufficient at maximal work, the O_2 tension of arterial blood should decrease and the CO_2 tension increase. The results show that the arterial CO_2 tension tends to decrease during exhaustive work. As the CO_2 transport by the lung is a function of alveolar ventilation it may be concluded that ventilation maintains a sufficient CO_2 transport between blood and ambient air, and also that alveolar hyperventilation often is present during heavy and exhaustive work. The O_2 tension decreased during heavy and ex-

haustive work but on an average only to 78.8 mm Hg (Table III), corresponding to an O_2 saturation of the arterial blood of 94 per cent (RAHN and FENN 1955). The decrease in the arterial O_2 tension during the large O_2 uptake of the heavy and the exhaustive work indicates that the O_2 exchange by the lung has a higher resistance to overcome than the CO_2 exchange. As ventilation is not limiting for the transport, the main O_2 tension gradient occurs in the exchange between alveolar air and blood. The diffusing capacity of the lungs in normal subjects usually seems to be larger than necessary for maximal O_2 uptake (LINDERHOLM 1959) but the O_2 diffusion gradient may, together with low ventilation-perfusion or low diffusion-perfusion relationships account for the slight decrease in arterial O_2 tension and O_2 saturation during heavy and exhaustive work. With increasing intensity of work and increasing O_2 uptake the circulation reaches its maximal capacity (maximal pulse frequency) before a decrease in oxygen saturation of significance for the O_2 transport is observed (see Fig. 1) in this material. This fact makes it justified to regard circulation and not lung function as the main limiting factor for O_2 transport, presuming that a muscular limitation of the working capacity is not present. Although muscular limitation cannot be excluded, it seems less probable in this material consisting of subjects particularly trained for cycling.

The increase in exercise tolerance during breathing of 60—100 per cent O_2, which is described by several authors (ASMUSSEN and NIELSEN 1946, BANNISTER and CUNNINGHAM 1954) may be explained by a complete O_2 saturation of the arterial blood and an increased amount of O_2 physically dissolved in the blood. In this material the O_2 content of arterial blood should be expected to increase 2—3 ml/100 ml blood during oxygen breathing. If the cardiac output was 25 l/min this increase might account for a 500—750 ml increase in O_2 uptake per min.

It seems probable that the material chosen includes subjects with a larger capacity for gas exchange than found in the average population.

Summary.

1. Oxygen and carbon dioxide tensions of arterial blood were determined in 14 young athletes, at rest and during muscular work of defined absolute and relative intensities.

2. The oxygen tension of arterial blood was lower during heavy work (pulse-rate 168) than at rest, the mean difference being 17 mm Hg. Exhaustive work caused a further decrease in O_2 tension (mean 7 mm Hg). Carbon dioxide tension tended to decrease during exhaustive work, and was remarkably low in some cases.

3. The divergent results reported earlier were probably obtained at different relative work loads. The necessity to define the work, at which measurements of arterial gas tensions are made, is stressed. The results are discussed with regard to the factors limiting the aerobic working capacity.

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Chemical Control of the Distribution of the Pulmonary Blood Flow.

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The question whether some mechanism exists, by which the relation between ventilation and perfusion of the lung alveoli is regulated, has been lively discussed for some time. Though it had been observed by PLUMIER (1904) and by BEYNE (1942) in the dog that lowering of the oxygen tension of the inspired air may lead to increased pulmonary artery pressure (PAP), the possibility that this might be an indication of such a regulating mechanism was first emphasized by EULER and LILJESTRAND (1946). In the anesthetized cat they found that breathing pure oxygen led to a lowering of PAP, whereas a gas mixture containing 10—11 per cent oxygen raised it considerably. Since the left atrial pressure (LAP) decreased somewhat, a fairly large rise in the gradient across the pulmonary vascular bed resulted, that could hardly be due mainly to increased cardiac output. The effect remained after bilateral vagotomy or the extirpation of the stellate ganglia. A similar though less pronounced pressure rise was observed after increasing the alveolar carbon dioxide tension. It was concluded that oxygen want and to a smaller degree accumulation of carbon dioxide leads to local contraction of pulmonary blood vessels, thereby causing a redistribution of the blood flow from insufficiently aerated to better ventilated parts of the lungs. Later experiments by different authors on this subject have been per-

formed on whole animals and on man as well as on isolated perfused lungs. The results will be briefly reviewed. The numerous reports concerning diseased subjects, however, fall outside the scope of this paper.

LOGARAS, working with cats (1947), confirmed the main results of EULER and LILJESTRAND, adding some further information. Sometimes the substitution of oxygen for air led to a great drop in PAP, presumably because respiration and circulation were impaired (cf. LILJESTRAND 1954). If oxygen want was induced in a spontaneously breathing cat by attaching a rubber tube to the tracheal cannula, practically the same rise in PAP was obtained whether the carbon dioxide was absorbed or not. Accumulation of carbon dioxide, taking place simultaneously with corresponding unsaturation of oxyhemoglobin, therefore plays a rather insignificant role for the rise in PAP in comparison to the effect of low oxygen tension itself. A rapid increase in PAP in the cat after inhalation of 8 per cent oxygen has also been demonstrated by LEUSEN and DEMEESTER (1955).

In the dog the results are conflicting. Some authors have found no increase at all of PAP at low oxygen tensions, others have observed a moderate rise that could be fully explained by the greater cardiac output, and some have got evidence of a definite increase in the pulmonary vascular resistance (PVR). The situation is complicated by the fact that determinations of the cardiac output during hypoxia from the gas exchange according to the often used FICK principle is apt to give too low values, as stated by NAHAS, HADDY and VISSCHER (1952, 1953), as well as by LEUSER and DEMEESTER. The difficulties may be illustrated by the following example. NAHAS *et al.* (1951) on 10 unanesthetized dogs with angiostomy cannulae in the pulmonary artery and the left auricle found that 8 per cent oxygen did not alter LAP but regularly increased PAP, on an average 28 per cent. Since cardiac output by the gaseous method decreased 30 per cent, it was concluded that the rise in PAP "truly results from a vasomotor change taking place in the pulmonary vascular bed". The following year the work of NAHAS, HADDY and VISSCHER (1952) led to the statement "that the FICK principle presupposed a nonexistent constancy of blood flow or A-V difference during the time of sampling". Using the dye dilution and the pulse contour methods NAHAS *et al.* (1954) found that the increased cardiac output could account for the rise in PAP without postulating an increased PVR. The same conclusion was arrived at by AVIADO *et al.* (1952), and AVIADO, LING and SCHMIDT (1957) point out that several opposing factors are responsible for the variable effect of hypoxia on PVR.

In the experiments of LEWIS and GORLIN (1952) PVR usually rose in dogs exposed to 10 per cent oxygen, if the oxygen saturation was above 55 per cent. Up to 3 hours the increase was small, while still longer periods were associated with more pronounced increase. The cardiac output, measured with the FICK method, which was felt to be applicable in these cases with long exposure and integrated simultaneous measure-

ments of A-V differences and oxygen consumption, changed only a little. If the hypoxia was greater (oxygen saturation below 55 per cent), there was uniformly a rise in cardiac output and a fall in PVR. BEARD, ALEXANDER and HOWELL (1952) in contrast to the observations quoted saw PAP essentially unaltered after inhalation of 8.5 per cent oxygen for 30 minutes, in spite of a slight increase in cardiac output. With still lower percentage of oxygen or with pure nitrogen they observed a rise in PAP. Since cardiac output was not determined, it is not possible to say whether an increase in PVR occurred. STROUD and RAHN (1953), using the FICK method, calculated an increase in PVR of 25 and 48 per cent, when the inspired oxygen concentration decreased from 21 to 15 and 8 per cent, respectively, but no change when the carbon dioxide content of the inspired air of the spontaneously breathing animal was raised to 5 per cent. Later STROUD and CONN (1954) obtained similar results from hypoxia, but this time radioactive K^{42} was used for determinations of the blood flow: the cardiac output during air breathing was on an average 3.79, with 10 per cent oxygen 4.01 and with 5 per cent oxygen 4.37 l per minute. The corresponding values for PAP were respectively 13.8, 20.4 and 22.4 cm water. RIVERA-ESTRADA *et al.* (1958) have observed a rise (of 4—5 mm Hg) in PAP in the dog during hypoxia.

In normal man MOTLEY *et al.* (1947) could rapidly induce pulmonary hypertension, if he breathed 10 per cent oxygen. Cardiac output, determined according to FICK, was found to fall slightly during hypoxia, so that the calculated PVR was almost doubled. A review of the original data in the light of new experiments indicated, however, that the subjects had not been in the "steady state" (FISHMAN *et al.* 1952). The cardiac output was now considered to increase in proportion to the hypoxia, but the increase was never in normal man enough to explain the hypertension which accompanied it (COURNAND 1950). Objections against the determinations of the cardiac output might possibly be raised with regard to the experiments of WESTCOTT *et al.* (1951), who found an average rise of PAP of 24.6 per cent in 27 mostly normal subjects during inhalation of 13 per cent oxygen, as well as those of SIEBENS, SMITH, and STOREY (1955). DOYLE, WILSON and WARREN (1952), however, made use of the dye dilution method. In 8 normal individuals they observed a rise of PAP (after 10 per cent oxygen) from 10 to 15 mm Hg, thus 50 per cent, while the cardiac output increased 3.4 to 4.4 (29 per cent) l per minute and sq. m. In contrast to these findings are the results of BOLT, VALENTIN and TIETZ (1957). Rebreathing (with absorption of carbon dioxide) about 10 minutes to 8—10 per cent oxygen led to a lowering of PAP, in spite of a large increase in the cardiac output: the FICK method gave an average of 5.5 l per minute before and 9.5 l at the end of the hypoxia. The authors believe that their results were due to the high age of their subjects.

It seems natural to assume that it might be possible to demonstrate the supposed role of oxygen want for the distribution of the pulmonary blood flow, if one lung is breathing a gas mixture poor in oxygen, whereas the other respires air or oxygen. One would then expect that

part of the blood flow will be shunted from the hypoxic to the other lung. Several authors have made experiments along these lines. Thus DIRKEN and HEEMSTRA (1949 a, b) in the anesthetized rabbit with one lung breathing a gas of low and the other one of high oxygen content found that the initial fall in oxygen saturation of the arterial blood became compensated to a major degree, but only after about 8 hours. The relative blood flow to a lung with low oxygen tension could thus be reduced to less than half of its original value, while the other showed a corresponding increase. The effect was not mediated by extrapulmonary nerves (DIRKEN and HEEMSTRA 1949 c). The authors conclude that a gradual increase in circulatory resistance of the hypoxic lung took place, caused by a local action of the low oxygen tension. Even at an alveolar oxygen concentration of approximately 12 per cent a distinct reaction occurred.

ATWELL *et al.* (1951) in three of six dogs within 20 minutes observed a well-marked shift of pulmonary blood flow toward the air-breathing lung and away from the other that was rebreathing air (with absorption of carbon dioxide). In the two of the reacting animals where PAP was measured, it increased during the rebreathing period. Blood flow was determined according to FICK, which introduces some uncertainty. The same applies to the findings of PETERS and ROOS (1952) who obtained similar results in 8 of 10 dogs. The calculated PVR increased in the hypoxic lung 1.3 to 4.5 times. These animals reacted with a significant rise in PAP, if both lungs breathed 10 per cent oxygen. No such rise was observed in two dogs whose ratio of blood flow remained unchanged. RAHN and BAHNSON (1950, 1953) have calculated the blood flow from each lung according to a modified FICK method. When the right lung was maintained on 30 per cent oxygen and the left lung was made hypoxic, there was a local constriction in the latter which increased with the degree of unilateral hypoxia. With the right lung maintained on air the left lung vessels constricted less for a given alveolar oxygen tension. BORST *et al.* (1957) have also demonstrated a vasoconstrictor effect on the hypoxic lung vessels of dogs with unilateral hypoxia, but as a rule the reaction became positive only after several hours.

In experiments on human subjects by BLAKEMORE, CARLENS and BJÖRKMAN (1954), both lungs were rebreathing, the one from an oxygen-rich gas mixture, the carbon dioxide being absorbed, the other from a gas mixture containing at the start about 5 per cent oxygen and 5 per cent carbon dioxide. In 8 of 9 patients they observed a redistribution of the pulmonary blood flow with a decrease in the hypoxic lung. Similar results were obtained by HERTZ (1955) in 6 of 8 cases — in the remaining 2 there was already before the trial a reduced blood flow in the lung concerned. The experiments of FISHMAN *et al.* (1955), where one lung respired 25 and the other 10 per cent oxygen, were quite negative, nor was there any alteration of PAP. Later, the effects of giving one lung 8 or 6 per cent of oxygen were studied in a few subjects (COURNAND 1957). Only in one of them was there a diminution of the flow through the hypoxic lung. COURNAND (1955) logically concluded that if these observations are confirmed, "doubt is cast upon the probable operation

in man of a well-controlled mechanism relating alveolar ventilation and perfusion".

Recently SVANBERG and COLLDAHL (1958) have determined the exhalation of acetylene through each lung after intravenous injection of a saline solution containing the gas. When one lung respired nitrogen and the other air, they could demonstrate a reduction of the giving off of acetylene from the hypoxic lung in 8 of 10 cases.

When changing only the carbon dioxide pressure in one lung, RAHN and BAHNSON (1953) observed no redistribution of the blood flow, but the mean error in the determinations is considerable, so that an effect cannot be excluded. According to PETERS (1957) unilateral breathing of carbon dioxide rich gas mixtures in the dog caused a lowering of PVR in that lung. HERTZ (1956), on the other hand, observed in 27 of 32 experiments on man some decrease of the relative blood flow through the lung that rebreathed an oxygen-rich gas mixture without absorption of carbon dioxide.

Studies on the isolated and perfused lungs have supplemented the picture. In such experiments the addition of carbon dioxide to the ventilating gas increased PVR, as shown by LÖHR (1924), BINET and BOURLIÈRE (1941), NISELL (1948, 1950, 1951 a and b), HEBB and NIMMO-SMITH (1948), and DUKE (1949, 1950, 1951). Similar results were obtained by BEAN *et al.* (1951), when "high carbon dioxide blood" was substituted for normal blood as perfusion fluid. The constriction of the pulmonary vascular bed when ventilating with a gas mixture with low oxygen concentration under corresponding circumstances was demonstrated by NISELL (1. c.), DUKE (1951, 1954, 1957), as well as by DUKE and KILLICK (1952 a and b). HÜRLIMANN and WIGGERS (1950), perfusing one lung lobe of a dog under constant pressure from the animal's carotid artery, obtained a reduction of the blood flow through the lobe during progressively developing hypoxia, in contrast to earlier experiments by BEYNE (1942).

If the perfusing blood had been made hypoxic or hypercapnic in an oxygenator before entering the lungs, NISELL (1951 a) observed a reduction of PVR. He concluded that the constriction of the vessels following ventilation with a gas mixture deficient in oxygen or with increased carbon dioxide content occurs in the venules or veins. HALL (1953), using the same technique as HÜRLIMANN and WIGGERS, found — in contrast to AVIADO *et al.* (1952) — a definite increase in PAP in a lobe perfused with oxygenated blood, when the lobe was ventilated with nitrogen instead of air. The site of action must therefore be in the capillaries or beyond. The conclusion is supported by the finding of NISELL (1951) of an increased lung volume (in agreement with the results of BINET and BOURLIÈRE 1941). DUKE, on the other hand (1951), did not observe any or only an insignificant increase in lung volume, but she confirmed (1954) that lowering the alveolar oxygen tension was a more effective way of influencing PAP than lowering the oxygen tension of the blood. She consequently considered the effect of oxygen want to be localized to the capillaries or the veins. This view is strengthened by the results of RIVERA-ESTRADA *et al.* that oxygen want in the

dog not only increased PAP but also — and to a still greater extent — the wedge pressure and the pressure in the pulmonary veins. Even if no increase in the wedge pressure has been found in man during hypoxia (DOYLE *et al.*), the evidence so far strongly supports the conclusion that the point of attack of oxygen want is to be sought in the pulmonary venules.

With regard to the mode of action of hypoxia local liberation of hormones (e. g. histamine) has been considered, but nothing definite is known. The possibility that increased carbon dioxide tension and reduced oxygen tension may act through the same mechanism, *e. g.* through changes in pH of the blood, has been discussed by NISELL (1948, 1950). For several reasons it seemed desirable to reconsider the question.

Methods.

The experiments were performed on the isolated and perfused lungs of the cat. In all 11 different experiments were made. The animals were anesthetized with chloralose (0.05—0.06 g per kg intravenously), cannulae were introduced into the trachea and the carotid arteries, and artificial respiration with positive pressure from a STARLING pump was started. The thorax was opened, the pulmonary artery prepared and a thread passed around it. The animal was then heparinized and bled through one or (better) both carotids. Cannulae were rapidly introduced into the pulmonary artery and the left auricle, the heart was tied off and the perfusion begun. The DALE-SCHUSTER pump that was used throughout had been filled during the final preparation of the animal with blood from it or from another cat. The blood was usually only diluted with about 1/5 to 1/4 of its volume with Ringer's solution, but since blood from two cats was mixed, some of it could be reserved as a store to replenish the circulating volume after the withdrawal of repeated samples. The plastic tube to the pulmonary artery was connected with a strain gauge manometer. In some experiments two parallel plastic tubes were connected with the outlet of the DALE-SCHUSTER pump and the pulmonary artery cannula respectively in such a way that the flow could be rapidly changed from one tube to the other, if it was desirable to perfuse with a limited amount of specially prepared blood (see expts 10 and 11). The blood from the lungs passed a recording pH-meter, described in detail by BJURSTEDT (1946) and kindly put at the author's disposal by him. Controls were often performed with samples obtained from the venous outflow and tried in a BECKMANN pH-meter. Samples for determinations of lactic acid were taken in the same way. They were centrifuged, and the plasma was analyzed for lactic acid with the aid of lactic acid dehydrase according to the method described by PFLEIDERER and DOSE (1955). The analyses were kindly performed by Dr. B. NORBERG. Injections were made into the tube leading to the pulmonary artery close to this. The perfusion pressure and the pH values were recorded on a GRASS polygraph. Different gas mixtures from containers were filled into rubber bags to be connected with the STAR-

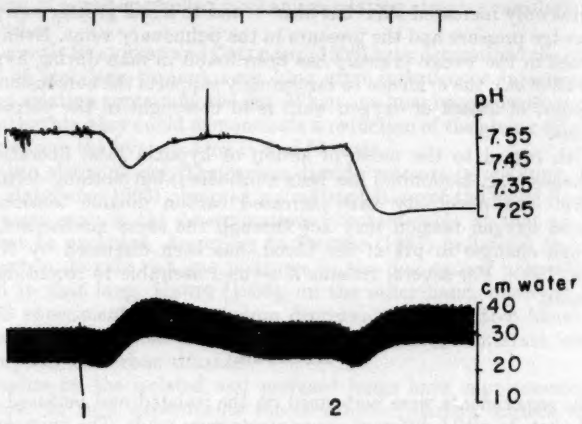

Fig. 1. Expt. no 10. Cat, 3.5 kg. In this and the following figures the records from above represent: Time in minutes, pH of blood after passing through lungs and perfusion pressure.

At start ventilation with air. At 1 perfusion with 30 ml of blood, acidified with 2—3 m.equiv. of lactic acid. This is immediately followed by the usual perfusion. At 2 ventilation with 5 per cent CO_2 in air.

LING pump. In order to keep the reaction of the perfusing blood fairly constant it was desirable to use gas mixtures containing 5 per cent carbon dioxide in air or oxygen between the tests.

Results.

As pointed out by earlier investigators a relatively high pressure is needed at the start of the perfusion of the isolated lungs. The reason is unknown, but the fact may presumably be ascribed to some functional derangement inflicted by the preparation (production of lactic acid?). Usually after about half an hour the pressure has gone down considerably and become stable enough to permit comparative tests of moderate duration. As mentioned above it is of value to avoid continuous loss of carbon dioxide from the blood with a consequent rise in pH and a decrease of the perfusion pressure by using gas mixtures containing 5 per cent carbon dioxide in the intervals.

The paramount importance of the carbon dioxide tension for the state of contraction of the pulmonary vessels is illustrated in Fig. 1.

Table I.

Effect of CO_2 on perfusion pressure and pH of perfused blood.

Expt. no.	Test	Perfusion pressure in cm water				pH of blood			
		before test	during test	percentage difference		before test	during test	difference	
3	Shift from air to 5 % CO_2 in air	20	31	+55		7.47	7.19	—0.28	
3	» » 5 % CO_2 in air to air	31	20		—36	7.19	7.45		+0.26
3	» » air to 5 % CO_2 in air	20	28	+40		7.45	7.14	—0.31	
4	» » » » » » » » »	26.5	28.5	+ 7.5		7.50	7.38	—0.12	
4	» » » » » » » » »	28	35	+25		7.55	7.42	—0.13	
5	» » » » » » » » »	26	28	+ 7.7		7.47	7.43	—0.04	
7	» » 5 % CO_2 in air to air	30	21		—30	7.55	7.97		+0.42
7	» » air to 5 % CO_2 in air	22	30	+36		7.98	7.55	—0.43	
7	» » » » » » » » »	27	30.5	+13		7.95	7.54	—0.41	
7	» » 5 % CO_2 in air to air	30	22		—27	7.54	7.87		+0.33
9	» » » » » O_2 to same after dilution with oxygen	31	29		— 6.5	7.30	7.39		+0.09
9	After further dilution with O_2	29	26.5		— 8.6	7.39	7.42		+0.03
9	After still further dilution with O_2	26.5	26		— 1.9	7.42	7.50		+0.08
10	Shift from 5 % CO_2 in O_2 to same after dilution with O_2	20.5	18.5		— 9.7	7.48	7.53		+0.05
10	After further dilution with O_2	18.5	18		— 2.4	7.53	7.56		+0.03

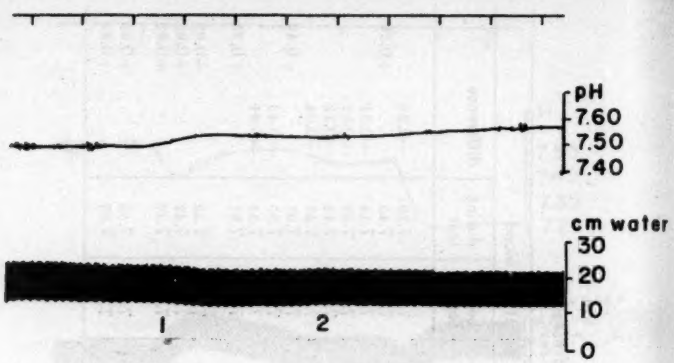

Fig. 2. Expt. no. 10. At start ventilation with 5 per cent CO_2 in O_2. At 1 O_2 is added to bag. At 2 more O_2 is added.

Soon after the substitution of 5 per cent carbon dioxide in air for air there is a decrease of the pH of the blood leaving the lungs, and within one or two minutes a steady state is reached. A corresponding rise in the perfusion pressure occurs, sometimes though not as a rule preceded by a fall as in Fig. 1. In Table I the results from a number of similar tests are given. It is obvious that the effect on the pH of the blood varies a great deal in the separate experiments. This is presumably due mainly to variations in the efficiency of ventilation and perfusion. For each preparation the agreement between the tests is fairly good, and the reversibility of the action of carbon dioxide is shown by a comparison between the changes induced in both directions, either from air to carbon dioxide in air or the reverse. There is a parallelism between the changes in pH and those of the perfusion pressure. The average rise in pH during a shift from 5 per cent carbon dioxide in air or oxygen to air or oxygen without any carbon dioxide is 0.33 and the average decrease when the shift takes place in the opposite direction is 0.25. The corresponding values for the percentage decrease or increase respectively of the perfusion pressure are 31 and 26.3. This gives about 1 per cent for a change in pH of 0.01. This great sensitivity can easily be demonstrated, if the carbon dioxide concentration of the ventilating gas mixture is stepwise reduced as in experiments 9 and 10 (Table I). Fig. 2 shows that a steady state was soon reached with the new gas mixtures.

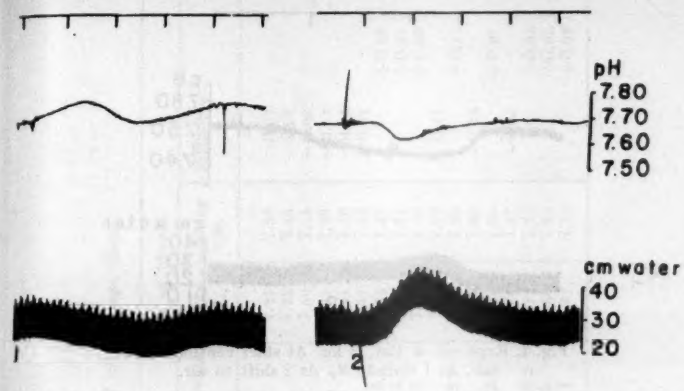

Fig. 3. Expt. no. 11. Cat, 3.5 kg. Ventilation with air. At 1 perfusion with 45 ml of blood, acidified with 1 m. equiv. lactic acid. At 2 perfusion with 20 ml of blood, acidified with 3 m. equiv. lactic acid.

Fig. 1 also illustrates that perfusion with a limited amount of blood that had been acidified by the addition of lactic acid, gave a response similar to that of carbon dioxide. But the figure seems to indicate a still greater sensitivity, since the drop in pH is small in comparison to the increase in pressure. It must be remembered, however, that the amount of acidified blood was rather small. Therefore part of the hydrogen ions must have been neutralized by the buffer action of the lung tissues, and no steady state had been attained. Under such circumstances the pH meter does not correctly reproduce the situation within the lungs and will give too high values. The conclusion is borne out by the following demonstration (Fig. 3). During ventilation with air at first 40 ml of blood that had been acidified with about 1 meq per l of lactic acid, were perfused. The pressure at first fell from 27 to 25 cm and then rose again to 28. The pH increased immediately after the perfusion and then fell from 7.73 to 7.65. In about 2 min it reached the original level again. Twelve min later 20 ml of blood containing about 3 meq per l of lactic acid were perfused. This time the pressure rose from 30 to 40 cm, whereas pH only decreased from 7.69 to 7.63 and in one minute attained its original level. Certainly this source of error may play a role also in other tests if the time for the test is too short, and may lead to erroneous conclusions. In most cases the rise in perfusion pressure with acidified

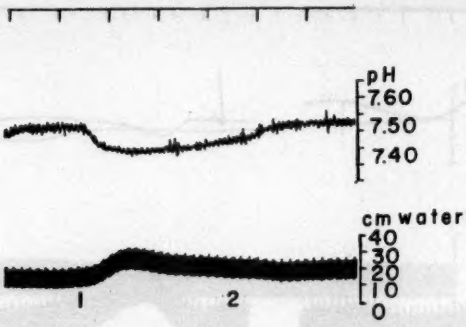

Fig. 4. Expt. no. 4. Cat, 3 kg. At start ventilation with air. At 1 shift to N_2. At 2 shift to air.

blood was preceded by a decrease which might have been the result of a dilatation of the arterioles as postulated by NISELL (1951 a) and by RIVERA-ESTRADA *et al.* (1958). In Fig. 1 the same occurred, however, also after shifting from air to air with 5 per cent carbon dioxide, though this was not usually the case.

Whereas increased carbon dioxide tension regularly causes a lowering of the pH and a rise of the perfusion pressure, this is not so with low oxygen tension. It sometimes happens that a preparation gives a similar response to ventilation with nitrogen as it does to increased carbon dioxide tension, though the effect of nitrogen is much smaller than that of 5 per cent carbon dioxide (Fig. 4). Sometimes the effect of hypoxia is the reverse, as illustrated in Fig. 5. Here a rise in the pH has occurred, but at the same time the perfusion pressure decreased. Table II summarizes the results when the ventilation was shifted between air and nitrogen. A strong effect of increased carbon dioxide tension (Table I) was usually accompanied by a fairly great sensitivity in the same direction for nitrogen ventilation, as seen in experiments 3,4 and 7, whereas in experiment 5 only a slight reaction for 5 per cent carbon dioxide and none at all for nitrogen occurs. In experiment 4 the two last tests with nitrogen were performed at a greater perfusion rate than was used in the first two and in the tests with carbon dioxide. The result of the increased blood flow was that the effect of hypoxia was very much reduced. A calculation analogous to that made for carbon dioxide shows that a change in pH of 0.01

Table II.

Effect of nitrogen ventilation on perfusion pressure and pH of perfused blood.

Expt. no.	Test	Perfusion pressure in cm water			pH of blood		
		before test	during test	percentage difference	before test	during test	difference
3	Shift from air to N_2 ventilation	18	23	+28	7.37	7.30	−0.07
4	» » » » » »	19	23	+21	7.52	7.45	−0.07
4	» » » » » »	19	21.5	+13	7.50	7.44	−0.06
4	» » » » » »	29	30	+ 3.5	7.43	7.39	−0.04[1]
4	» » » » » »	20	21	+ 5	7.41	7.40	−0.01[1]
4	» » » » » »	26	26	0	7.47	7.46	−0.01
5	» » » » » »	22	21	− 4.6	7.52	7.55	+0.03
6	» » » » » »	26	25	− 3.8	7.57	7.61	+0.04
6	» » » » » »	22	20	−10	7.47	7.50	+0.03
6	» » » » » »	21	27.5	+26	7.97	7.86	−0.11
7	» » » » » »	27.5	22.5	−18	7.86	7.99	+0.13
7	» » N_2 to air ventilation	22	28	+27	7.87	7.69	−0.18
7	» » air to N_2 »	23.5	23	− 2.1	7.88	7.95	+0.07
7	» » » » » »	26	28.5	+ 9.6	7.50	7.47	−0.03
9	» » » » » »	29	27.5	− 5.2	7.72	7.75	+0.03
9	» » » » » »	20	19	− 5	7.60	7.65	+0.05
10	» » » » » »	21	19.5	− 7	7.67	7.75	+0.08
10	» » » » » »						

[1] Perfusion rate increased.

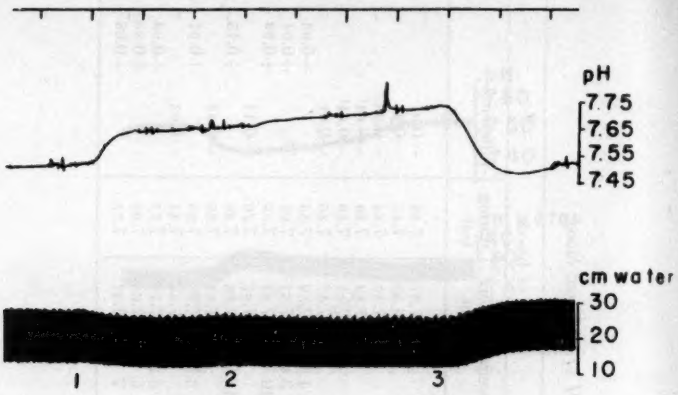

Fig. 5. Expt. no. 10. At start ventilation with O_2, containing some CO_2. At 1 shift to air. At 2 shift to N_2. At 3 shift to 5 per cent CO_2 in O_2.

corresponds to a change in perfusion pressure of about 2 per cent when the pressure rose and 1 per cent when it fell. The agreement is as good as can be expected under the circumstances.

A few experiments have been performed with ventilation with 5 or 10 per cent oxygen and a concentration of carbon dioxide of 5 per cent as compared to 5 per cent carbon dioxide in air or oxygen. The results, given in Table III, are in agreement with those of Table II. Since, however, small variations in the carbon dioxide concentration (of the order of 0.1 to 0.2 per cent) cannot be excluded in some of the gas mixtures that were compared to each other, this might have vitiated the results, and the method was abandoned. It should be pointed out that some observations of earlier authors seem to have been made without the risks having been clearly appreciated.

Duke and Killick have demonstrated (1952 b) that the pressure rise obtained during ventilation with nitrogen can be reversed by monoiodoacetic acid. I have repeated their experiment. The fact that monoiodoacetic acid often causes a continuous rise in the perfusion pressure involves a certain difficulty. By administering the drug in repeated small doses the direct effect could be avoided and the reversal confirmed (Fig. 6). Before the injection the perfusion pressure in consequence of the shift from air to nitrogen rose from 13.5 to 15 cm, after the injection of monoiodoacetic acid

Table III.

Effect of ventilation with 5 or 10 per cent O_2 on perfusion pressure and pH of perfused blood.

Expt. no.	Test	Perfusion pressure in cm water			pH of blood		
		before test	during test	percentage difference	before test	during test	difference
1	Shift from 5 % CO_2 in O_2 to 5 % CO_2 + 5 % O_2	27	29	+ 7.4	7.71	7.67	—0.04
1	» 5 % CO_2 in O_2 to 5 % CO_2 + 5 % O_2	33	34	+ 3	7.46	7.42	—0.04
2	» » 5 % CO_2 in O_2 to 5 % CO_2 + 5 % O_2	17.5	25	+43	7.34	7.20	—0.14
2	» » 5 % CO_2 in O_2 to 5 % CO_2 + 5 % O_2	23	25	+ 8.7	7.15	7.14	—0.01
3	» » 5 % CO_2 in air to 5 % CO_2 +10 % O_2	28	28.5	+ 1.8	7.14	7.10	—0.04

Fig. 6. Expt. no. 8. Cat, 3.8 kg. Ventilation at the start with air. At 1 and 2 ventilation with N_2. Between A and B injection of monoiodoacetic acid (45 mg).

ventilation with nitrogen led to a decrease from 13.5 to 13 cm. Unfortunately no determinations of the pH of the blood were made at the time.

Since ventilation with nitrogen sometimes leads to a decrease of the pH it was considered desirable to investigate, whether low oxygen tension might induce liberation of lactic acid in the lungs. Determinations on the blood from the lungs were made under varying conditions. The results, put together in Table IV, show that a continuous increase in the lactic acid concentration takes place, as the experiment goes on, even to a small extent during ventilation with air or oxygen. It seems reasonable to assume that this is due to the fact, that the nutritional vessels reach the lungs from the bronchial arteries which are not included in the perfusion as arranged in these experiments (cp. DALY 1958). But the liberation of lactic acid is usually greatly enhanced by ventilation with gas mixtures containing low concentrations of oxygen or pure nitrogen. Thus in experiments 3, 7 and 10 the rise in lactic acid concentration proceeds about 4—10 times as rapidly during ventilation with nitrogen as it does with air or oxygen. The two first mentioned experiments are also those where the largest decrease in the pH during ventilation with nitrogen took place. (In the last test with shift from air to nitrogen in experiment 7 no samples were taken.) But also in other experiments it seems probable that there has been an increased liberation of lactic acid during ventilation with nitrogen as well as with 5—10 per cent oxygen, though this has not always been enough to raise the acidity of the blood, since at the same time carbon dioxide was given off and oxyhemoglobin partially reduced.

Table IV.

Effects of varying oxygen tensions on lactic acid in perfused blood.

Expt. no.	Test	Lactic acid concentration in plasma in meq per l		Time in min.	Increase in lactic acid concentration per min.
		before test	during test		
3	Shift from 5 % CO_2 in air to 5 % CO_2 + 10 % O_2	6.2	6.6	4	0.1
3	" air to N_2	7.1	7.9	3.5	0.23
4	" 5 % CO_2 in O_2 to 5 % CO_2 + 10 % O_2	9.2	9.3	5	0.02
4	" 5 % CO_2 in air to 5 % CO_2 + 5 % O_2	10.5	10.7	4	0.05
5	" air to N_2	10.3	10.5	8	0.025
7	" air to N_2	4.7	5.6	6	0.15
7	" N_2 to air	5.6	5.7	7	0.02
7	" air to N_2	7.3	8.2	3	0.29
9	" air to N_2	6.3	6.8	7	0.07
9	" N_2 to air	6.8	7.4	25	0.024
10	" air to N_2	[1]16.1	18.2	9	0.23
10	" N_2 to O_2 ($+CO_2$)	18.2	18.5	5	0.06

[1] The high initial value is due to a standstill of the perfusion during some minutes.

Discussion.

It has been found in this study that lowered oxygen pressure in the isolated and perfused lungs causes a liberation of lactic acid. Hereby the pH of the perfused blood may decrease. A certain parallelism has been observed between this decrease and the rise in PAP. If only a very small rise in the lactic acid concentration is produced, hypoxia may have no effect at all or even lead to a lowering of PAP, but in this case a shift of the blood pH in the alkaline direction takes place — presumably mainly due to the reduction of oxyhemoglobin. The fundamental role of lactic acid for the rise of PAP during hypoxia is further supported by the observation that if the liberation of it is stopped by monoiodoacetic acid, the pressor effect disappears or is reversed.

There is every reason to assume that the effect of carbon dioxide on the pulmonary vessels, leading to a rise in the perfusion pressure, is mediated by changes in pH, and this view is supported by the results of tests with acidified blood. The quantitative agreement between the effects of low carbon dioxide concentrations and of nitrogen leads to the assumption that both act in the same way and by the same mechanism on the isolated perfused lungs. Such a possibility has been discussed by NISELL (1948, 1950). Since he found that hypoxia and a rise of the carbon dioxide tension both caused a rise in PAP but changed the pH of the perfusing blood in opposite directions, he rejected the idea. In the only experiment quoted by NISELL in this connection, ventilation with nitrogen led to an increase of pH of 0.13, whereas the substitution of 8 per cent carbon dioxide in air for 2 per cent in air caused a decrease of 0.18—0.19. This latter figure is in harmony with the writer's observations, but NISELL's result with nitrogen ventilation is irreconcilable with them. The great rise in pH is difficult to explain. Unfortunately NISELL had administered 100 micrograms of carbaminoylcholine before the test, and it is unknown whether this has influenced his results.

DUKE and KILLICK (1952 b) have measured the pH of the perfusing blood during ventilation with air and with nitrogen. In one case there was no alteration, in the other five a decrease was obtained with nitrogen, on an average 0.05, which is in keeping with the writer's observations. They seem to attch no importance to their findings, however, possibly because the changes in pH

were relatively small and variable (cf. DUKE 1951). This is hardly astonishing, since the increase in lactic acid concentration and the desaturation of the oxyhemoglobin are dependent *i. a.* on the perfusion rate, which will have different quantitative effects on these two antagonistic factors. That even small alterations of pH will influence PAP is proved by the experiments with varying concentrations of carbon dioxide added to the ventilating gas mixture.

From the results obtained it seems plausible that the oxygen tension will be of importance for the regulation of the pulmonary blood flow, as indicated by EULER and LILJESTRAND. By local release of lactic acid a shift will be induced in the acid direction of the blood. This in its turn will cause a constriction of venules of that part, and the local blood flow will decrease. DUKE (1951, 1954) observed increased PVR when the lungs were ventilated with 10 per cent oxygen instead of air and a variable effect with 15 per cent oxygen. As pointed out above a rise in the lactic acid liberation probably occurred with 10 per cent oxygen. This would mean that already a moderate reduction of the alveolar oxygen tension might cause a redistribution of the pulmonary blood flow. Probably the release of lactic acid will proceed at a much quicker rate, if the oxygen tension drops further, as is known from experiments on the whole animal (HENDERSON and GREENBERG 1934).

It is interesting that if one leg is perfused in series with the lungs, ventilation with low oxygen percentage leads to a dilatation of the vessel of the limb, as demonstrated by BEYNE (1942) and by DUKE (1957). This is in keeping with the well-known fact that increased acidity of the blood causes a dilatation of the vessels of the extremities.

During physiological conditions variations in the carbon dioxide pressure within the lungs may occur, but their effect on the acidity of the blood are then as a rule nearly completely offset by simultaneous oxygenation of reduced hemoglobin or reduction of oxyhemoglobin. It is therefore evident that accumulation of carbon dioxide will then be of small importance for the control of the pulmonary vascular bed. The situation may be entirely altered however, if a general change in the carbon dioxide tension in the body is induced without the corresponding change in hemoglobin. Such is the case during overventilation and also, if carbon dioxide is added to the inspired air without eliciting a sufficient increase in ventilation.

Experiments on the isolated and perfused lungs are well suited to studies of local regulating mechanisms, and there can be little doubt that these must function also in the intact body. In the whole animal, on the other hand, different factors, such as changes in cardiac output, hormonal and nervous influences may be operating and modify the results (cf. DALY 1958). Special emphasis must also be attributed to changes in the acid-base balance. In the following an attempt will be made to analyze some of the conflicting results of earlier authors from this point of view.

Lowered oxygen tension stimulates respiration and thereby induces washing out of carbon dioxide. This might well explain, why NAHAS *et al.* (1954) were unable to find increased PVR in dogs breathing 8 per cent oxygen. It is mentioned in their paper that a steady state had not developed when the determinations were made. It is not improbable that ventilation may become more affected if the animals are awake than if they are narcotized. In accordance with this are the results of STROUD and RAHN (1953) and STROUD and CONN (1954). That the first mentioned authors found no rise in PVR, if the animals were breathing 5 per cent carbon dioxide is not unexpected, since the alveolar carbon dioxide tension may be only slightly increased with a change in the opposite direction of the alveolar oxygen tension. One would even expect that a considerable overventilation might lead to a fall in PAP, if it is not completely compensated by liberation of lactic acid. This would seem to be the case in the elderly patients of BOLT *et al.* (1957) where the cardiac output was also greatly increased.

Especially puzzling has been that an expected redistribution of part of the blood flow from one lung breathing a gas mixture with a low to the other with a high oxygen percentage has only been found under special circumstances. Thus in man sometimes no effect appeared after 10 per cent or even less, whereas 12 per cent given on both sides led to an increased PAP. As a matter of fact, however, the experiments with different gas mixtures for the two lungs give rise to complicated alterations of the blood pH. Under ordinary conditions the respiratory gas exchange has only a small effect on the reaction of the blood, the loss of carbon dioxide being nearly completely balanced by the oxygenation of reduced hemoglobin. The pH of the arterial blood is therefore usually only 0.02—0.04 higher than that of the mixed venous blood. But with oxygen pressure high in one lung and low in the

other the influence of the gas exchange will be quite different on the two sides. In the blood passing the alveoli with low oxygen pressure the loss of carbon dioxide will be incompletely compensated by the relatively small oxygen uptake, so that the capillary blood will become more alkaline than normally; in the other lung the reverse holds true. From the experiments of FISHMAN *et al.* (1955) it is evident that this has happened, since the respiratory quotient rose in all experiments on the side breathing 10 per cent, whereas it decreased with 25 per cent oxygen. The difference between the two sides is probably about half the normal difference between mixed venous and arterial blood, thus 0.01—0.02. On the other hand a certain amount of lactic acid will have been liberated on the hypoxic side, and this will cause a lowering of the pH, the degree of which being of course also dependent on the blood flow. The final outcome will be determined by the relative importance of the two factors influencing the pH of the blood, *i. e.* the respiratory gas exchange and the local release of lactic acid. If the net result is that the pH of the blood passing the pulnonary venules on both sides is about the same, then no redistribution of the flow will take place, if it is higher in one lung, then more of the flow will be shunted over to that side. In the experiments of FISHMAN *et al.* the cardiac output was 7.69 l per min or 33 per cent above the predicted figure for normal resting individuals, which will tend to lower the concentration of lactic acid in the blood. Assuming that the liberation of lactic acid goes at a much quicker rate at low than at moderate oxygen pressures and overcompensates the effect of the reduction of oxyhemoglobin, one must except that it will be possible to demonstrate the shunting from one lung to the other, if lower oxygen concentrations than 10 per cent are tried. This is the case in the experiments of SVANBERG and COLLDAHL and probably also in those performed by HERTZ and by BLAKEMORE *et al.* An accumulation of carbon dioxide in the experiments of the last mentioned authors may also contribute to their positive results.

Obviously reduction of the general blood flow will increase the concentration of lactic acid at the site of its liberation. Since it seems reasonable to assume that the general blood flow in the anesthetized rabbit might have slowed down gradually in the course of many hours, this might well lead to an alteration of the ratio between the flow through the two lungs as in the experiments of DIRKEN and HEEMSTRA. With regard to the "time factor" observed

by BORST *et al.* this explanation cannot be applied, for the blood was driven at constant speed through the lungs by a pump. But after passing the lungs it was also circulated through the body of the animal, and this might involve unknown complications owing to alterations of the blood induced by the hypoxia. In this connection it is interesting to note that the answer of the pulmonary vessels to hypoxia may be influenced *e. g.* by the adrenal glands and by the sympathetic innervation (NAHAS, MATHER and KITCHELL 1953, NAHAS *et al.* 1954, DIRKEN and HEEMSTRA 1949 c).

Summary.

1. The constriction of pulmonary vessels obtained on the isolated and perfused lungs of the cat by raising the carbon dioxide tension of the ventilating gas mixture is mediated by lowering of the pH of the blood, a decrease of 0.01 corresponding to a rise in perfusion pressure of about 1 per cent. Ventilation with nitrogen sometimes leads to a decrease and sometimes to an increase in the perfusion pressure. There are parallel changes in the pH, so that it rises in the former and drops in the latter case. The quantitative relations between the changes in the pH and perfusion pressure correspond to those found with varying concentrations of carbon dioxide. Lactic acid is continuously given off from the perfused lungs; but the liberation is greatly enhanced by hypoxia. Injections of monoiodoacetic acid abolishes or reverses the pressure rise obtained during ventilation with nitrogen.

2. It is concluded that hypoxia acts on the pulmonary vessels by liberation of lactic acid and thereby may cause a local regulation of the relation between blood flow and ventilation. The consequences of these findings for the interpretation of the results of some earlier authors are discussed.

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The Effect of Ethyl Alcohol on the Secretion from the Adrenal Medulla in Man.

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While the effects of ethyl alcohol on the central nervous system have been extensively studied, the relation between alcohol and the autonomic nervous system has received less attention. It has, however, been shown by various investigators that alcohol may exert an effect on several organs with an autonomic nerve supply. The cutaneous vasodilation occurring in man after moderate amounts of alcohol is well known. MENDELOFF (1954) found that alcohol increased liver blood flow in man, and LASKER, SHERROD and KILLAM (1955) reported that alcohol caused an increase in the coronary blood flow in dogs. The general action of alcohol in moderate amounts on the circulation in man is, however, considered to be slight (GOODMAN and GILMAN 1955, pp. 99—100).

Until recently the effect of alcohol on the adrenal medulla has apparently not been studied. A method for obtaining information about the activity of the adrenal medulla has evolved from the study of the urinary excretion patterns of adrenaline and noradrenaline in patients before and after adrenalectomy. From this and other studies by EULER and co-workers (cf. EULER 1956, pp. 285—289) it is evident that most of the urinary adrenaline comes from the adrenal medulla while the noradrenaline is chiefly derived from the vasomotor nerve endings.

KLINGMAN and GOODALL (1957), using this method, studied the effect of alcohol on the activity of the adrenal medulla in dogs. They found that large amount of alcohol produced an increase in the urinary output of adrenaline and a reduction of the adrenaline content of the adrenal gland, indicating a strong activation.

In the present investigation the effect of moderate amounts of alcohol on the adrenal medulla was studied in man using the urinary adrenaline excretion as an index of the secretory activity of the medulla.

Material and Methods.

Healthy male students were used as test subjects. The experiments were performed between 07.00 and 16.00. The subjects had, as a rule, been fasting since the previous evening.

Urine was collected during two consecutive 4-hour periods, the first starting between 07.00 and 08.00. At the beginning of the second period the subject was given a moderate or low quantity of alcohol to drink in 20 minutes. The blood alcohol concentration was followed at regular intervals for a period of 5—7 hours. Food was not allowed until $2^1/_2$ hours after alcohol intake in order not to influence the first part of the blood alcohol curve. Smoking was, as a rule, prohibited during the experiments. 43 alcohol experiments were performed on 13 subjects. In addition 23 control experiments without alcohol were carried out on the same subjects.

In a few alcohol and control experiments the subject was not fasting and was allowed to smoke moderately during the whole experiment.

The alcohol was given as whisky (43 per cent by volume) or as wine (10—12 per cent by volume) in a dose of 0.27—0.54 g ethanol per kg. The peak blood alcohol level varied between 0.015 and 0.080 per cent.

The blood alcohol concentration was determined with the Widmark method (1932) in triple samples.

Urine analysis of adrenaline and noradrenaline was carried out with the fluorimetric method of EULER and FLODING (1956). The excretion figures were expressed in mμg/min. The diuresis was noted in each experiment.

Results.

Table I shows the mean excretion figures for adrenaline and noradrenaline in the alcohol and control experiments. There is good agreement between the values from the first 4-hour periods in the two different groups. In Fig. 1 the adrenaline values of the first and the second 4-hour period are plotted against each other. The variability is considerable, but the tendency to increased adrenaline values after alcohol intake is noticeable.

Table I.

Mean figures for the urinary excretion of adrenaline and noradrenaline in 43 ethyl alcohol and 23 control experiments.

	Adrenaline mμg/min (M. ± S. E.)		Noradrenaline mμg/min. (M. ± S. E.)	
	First 4-hour period	Second 4-hour period	First 4-hour period	Second 4-hour period
Control experiments (n = 23)	4.7 ± 0.32	5.2 ± 0.51	14.5 ± 1.66	16.0 ± 1.76
Alcohol experiments (n = 43)	4.8 ± 0.38	8.0 ± 1.58	13.5 ± 1.16	17.0 ± 0.82

In each experiment the difference between the adrenaline excretion in the two 4-hour periods was computed and used for the statistical evaulation. Thus every subject served as his own control.

A slight increase of 0.5 mμg/min during the second 4-hour period in the control experiments was found not to be statistically significant ($p > 0.05$). A statistically highy significant increase of 3.2 mμg/min ($p < 0.001$) was obtained, however, in the alcohol experiments, corresponding to an increase in the adrenaline excretion of 67 % above normal.

Fig. 2 shows the differences between the second and the first 4-hour periods in each alcohol experiment in relation to the peak blood alcohol level attained in the same experiment. No systematic relation between the increase and the peak blood alcohol level can be seen.

The noradrenaline excretion was mainly unchanged in the alcohol experiments as well as in the controls (Fig. 3).

The values obtained when the subject was not fasting and when moderate smoking was allowed during the experiment were in accord with the rest of the results.

Increased diuresis was frequently noted in the alcohol experiments. The diuresis showed considerable individual variation and was in some test subjects not affected by alcohol intake.

As a rule no gross signs of alcohol intoxication were noted in the test subjects.

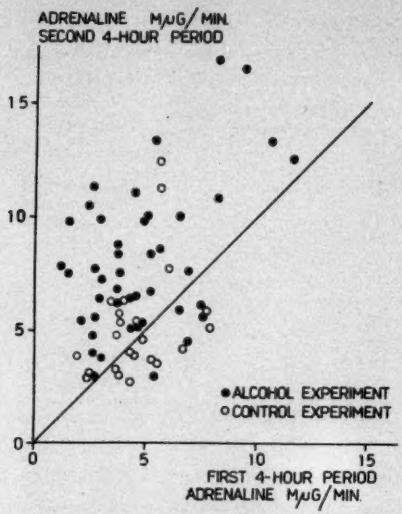

Fig. 1. Effect of alcohol on the urinary excretion of adrenaline. The urinary adrenaline excretion in the first 4-hour period plotted against the value obtained in the second 4-hour period.

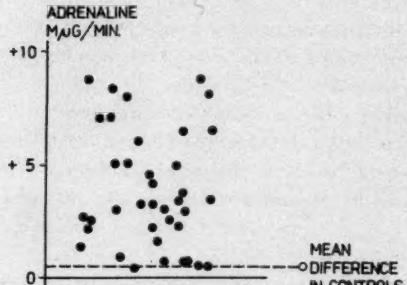

Fig. 2. Change in urinary adrenaline excretion after alcohol intake related to peak blood alcohol level.

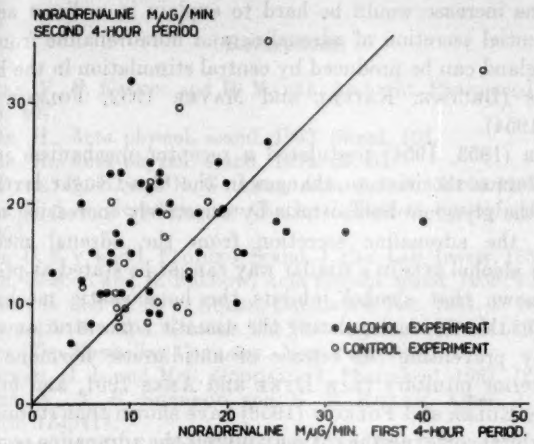

Fig. 3. Effect of alcohol on the urinary excretion of noradrenaline. The urinary noradrenaline excretion in the first 4-hour period plotted against the value obtained in the second 4-hour period.

Discussion.

The present experiments have shown a significant increase in the urinary excretion of adrenaline after ingestion of moderate doses of alcohol in man, while the noradrenaline excretion did not differ from that obtained in control experiments. The results suggest an increased adrenaline secretion from the adrenal medulla since most of the adrenaline in urine is known to originate from this organ. The unaffected noradrenaline output implies that the vasomotor system or the adrenergic system in general is not involved.

The diuresis was usually higher in the alcohol experiments but a comparison between the alcohol and control group gave no indication that a high diuresis in itself increases the adrenaline figures, nor was a concomitant increase observed in the noradrenaline excretion.

Alcohol could effect an increased secretion from the adrenal medulla either by direct action on the chromaffin cells in the gland or by central action mediated via the splanchnic nerves. The latter alternative seems to be more likely since a selective

adrenaline increase would be hard to explain in a direct action. A differential secretion of adrenaline and noradrenaline from the adrenal gland can be produced by central stimulation in the hypothalamus (BRÜCKE, KAINDL and MAYER 1952, FOLKOW and EULER 1954).

DUNÉR (1953, 1954) postulated a receptor mechanism in the hypothalamus sensitive to changes in the blood sugar level and serving the glycemic homeostasis by selectively increasing or decreasing the adrenaline secretion from the adrenal medulla. Whether alcohol acts in a similar way cannot be stated at present. It is known that alcohol inhibits the homeostatic mechanism located in this region regulating the osmotic concentration of the blood by preventing the release of antidiuretic hormone from the posterior pituitary (VAN DYKE and AMES 1951, and others). Recently EULER and FOLKOW (1958) have shown that stimulation of the orbital cortex in the cat may inhibit the adrenaline secretion selectively.

It is noteworthy that increased adrenaline secretion is found at peak blood alcohol levels as low as 0.02—0.04 per cent. The effect of alcohol on the adrenaline secretion might be of interest for the understanding of certain pharmacological actions of alcohol and seems to merit further study.

Summary.

1. The effect on the urinary excretion of adrenaline and noradrenaline of moderate amounts of alcohol which gave peak blood levels of 0.015—0.080 per cent was studied in healthy human subjects.

2. The urinary adrenaline excretion was found to increase significantly in the alcohol experiments when compared to control experiments. The increase was already noticeable when the peak blood alcohol concentration was below 0.04 per cent. No consistent change in the urinary noradrenaline excretion was observed after alcohol intake.

3. It is concluded that the increased adrenaline excretion is due to adrenal medullary stimulation. Some possible mechanisms for this effect are discussed.

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Effect of a Single Dose of Some Hormones on Plasma Unesterified Fatty Acid (UFA).

By

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Investigations which have shown that UFA in plasma increases in conditions associated with an increased transport of fat from the fat depots have resulted in the hypothesis that UFA constitutes an important form of transport of fat (DOLE, 1956 GORDON and CHERKES 1956, LAURELL 1956, SPITZER and MILLER 1956). Judging by investigations of the turnover rate of UFA in plasma, this is sufficiently high to satisfy the calorie requirements in carbohydrate deficiency (HAVEL and FREDRICKSON 1956, LAURELL 1957). Whether triglycerides are also of importance in this respect is not known.

That UFA in plasma is to some extent under endocrine control has been shown by DOLE (1956) and by GORDON and CHERKES (1956), who found that adrenaline produces a marked increase and insulin a decrease in the plasma UFA.

In the present investigation interest was focussed on the immediate effect on UFA of some hormones believed to be capable of mobilizing fat. In some cases the investigation included analysis of triglycerides and phospholipids.

Growth hormone (GH) has repeatedly been found to increase liver fat at the expense of depot fat in mice and rats (LI, SIMPSON and EVANS 1949, GREENBAUM and MCLEAN 1953). REISS (1947) claimed lactogenic hormone (LH) to have a fat-mobilizing

effect on the rat and on homo, an effect, which could not be confirmed by LI *et al.* (1949). Glucagon has also been described by CAVALLERO (1956) as possessing such an effect.

The above mentioned effect of adrenaline on UFA in plasma justified the inclusion of noradrenaline in the investigation. Finally, the effect of ACTH was studied to determine whether adrenaline in this respect acts via ACTH release.

Experimental. The experiments were carried out on apparently healthy volunteers of both sexes, aged 25—34 years and in the postabsorptive state.

All of the hormones were administered slowly by intravenous drip after dilution with 200—300 ml saline. Blood samples were collected with the same cannula as was used for the drip, after the latter had been interrupted for half a minute. EDTA-Na_2 was used as an anticoagulant.

Triglycerides were determined as formaldehyde after separation according to BORGSTRÖM (1952), hydrolysis and oxidation of the glycerol with periodic acid (CARLSSON and WADSTRÖM, personal communication), Standard deviation $\pm$ 1.1 mg/100 ml. Phospholipids were determined according to ALLEN (1940) after extraction with chloroform : methanol 2 : 1 with the use of the factor 25 for conversion from phosphorus to phospholipids. Standard deviation $\pm$ 1.5 mg/100 ml.

UFA in plasma was determined according to GROSSMAN *et al.* (1955). Standard deviation $\pm$ 0.019 meq/liter. This method has some inherent disadvantages. Determination of the yield of saturated aliphatic fatty acids added to serum has shown that fatty acids with less than 16 carbon atoms are not extracted quantitatively and those with less than 8 carbon atoms not at all (LAURELL 1956). The yield has also been determined on addition of oleic acid and linoleic acid, which were extracted to 100 and 97 per cent, respectively. Determination of the phospholipid content of the petroleum ether extract by the method of GROSSMAN from serum showed that this corresponds to 78 per cent of the total phospholipids. For this reason the error introduced by phospholipid groups titratable with alkali was determined (cephalines with ethanolamine and serine content are of interest in this respect: GARVIN and KARNOVSKY 1956.)

The UFA content of 2 ml serum was estimated by double determination according to GROSSMAN *et al.* (1956). Two petroleum ether extracts from the same serum were separated according to

Table I.

Distribution of titratable acidity between the phospholipid fraction and the non-phospholipid fraction in extracts prepared by the methods of Sperry and Brand (1955) and Grossman et al. (1955) from a serum containing 0.62 meq UFA per litre, estimated according to Grossman et la. (1955).

Extraction procedure	Non-phospholipid acidity meq/l	Phospholipid acidity meq/l
Grossman	0.50	0.12
	0.51	0.16
Sperry	0.58	0.14
	0.58	0.16

Borgström (1952) on silicic acid into the phospholipid fraction and the nonphospholipid fraction containing free fatty acids.

Extracts of 2 ml of the same serum with chloroform-methanol 2 : 1 was purified by the method of Sperry and Brand (1955) with M/30 KH_2PO_4 instead of water, a procedure not involving any loss of higher fatty acids (Wadström, personal communication). The purified extract was separated in the way described above.

The result of titration of these fractions dissolved in 3 ml ethanol with 0.02 N NaOH (thymol blue) are given in Table I.

It is apparent that losses of multiple unsaturated and lower fatty acids during extraction are approximatively compensated by the occurrence of titratable phospholipids in the extract.

In the present investigation the reproducibility of the determinations was more important than the absolute values and the lower degree of accuracy of the latter was not considered sufficient to justify the use of the more exact but much more complicated method described by Gordon, Cherkes and Gates (1957).

All standard errors were calculated on the basis of 15 determinations per serum.

Hormones used:

GH: Somacton, claimed to contain 0.5 u. s. p./mg[1].

LH: Prolactin[1].

ACTH: Acton[1].

l-noradrenaline: Noradrenaline, Byk-Gulden.

Glucagon: crystalline, kindly supplied by Eli Lilly and Co.

[1] Kindly supplied by Ferring AB, Malmö, Sweden.

Effect of administration of some hormones on UFA (meq/litre), glucose (mg/100 ml), glycerides (mg/100 ml), and phospholipids (mg/100 ml).

Exp. no.	Hormone dose	Infusion volume	Infusion time		Time (min) 0	15	30	45	60	75	90	120	135	150
		ml	min											
1	GH 75 u. s. p.	200	30	UFA	0.79	0.81	0.93	0.89	0.85	1.06	0.96			
2	LH 500 I. U.	200	45	Glucose	120	—	—	150	130	130	100			
				UFA	0.51	0.51	—	0.45	0.39	0.42	0.39			
				Glycerides	61	52	—	53	55	53	56			
				Phospholipids	206	190	—	186	180	185	184			
3	LH 500 I. U.	200	45	Glucose	110	110	120	110	110	120	110			
				UFA	0.60	0.53	0.54	0.50	0.54	0.58	0.58			
4	ACTH 10 I. U.	300	115	Glucose	120		110		110		120	120		120
				UFA	0.55		0.71		0.87		0.77	0.73		0.78
				Glycerides	110		96		89		92	100		110
				Phospholipids	275		252		223		223	233		238
5[1]	ACTH 10 I. U.	300	120	Glucose	90	90	80		90		90	90	90	90
				UFA	1.27	1.06	0.94		1.02		1.08	1.09	1.16	1.18
				Glycerides	69	61	61		61		59	59	57	56
				Phospholipids	235	200			203		207	200	198	208
6	Norepinephrine 1 mg	200	37	Glucose	110	120	150	130	110	—				
				UFA	0.77	1.84	1.45	1.12	0.57	0.43				
				Glycerides	57	59	64	55	55	56				
				Phospholipids	232	235	227	217	212	212				
7	Norepinephrine 1 mg	200	40	Glucose	110	110	120	130	120	120				
				UFA	0.42	0.69	0.72	0.94	0.53	0.39				
8	Glucagon 1.5 mg	150	40	Glucose	110	150		110	90	70	80			
				UFA	0.49	0.57		0.28	0.31	0.30	0.25			
				Glycerides	66	60		58	57	56	57			
				Phospholipids	245	217		220	217	215	212			
9	Glucagon 2 mg	200	35	Glucose	120	—	100	120	—	90	—	—		
				UFA	0.89	0.86	0.58	0.42	0.47	0.58	0.55	0.62		

[1] Experiment 5 carried out after starvation for 36 hours.

Results and discussion. The results are given in Table II.

The increase of UFA in plasma on administration of GH was not much greater than that observed during fasting by GORDON and CHERKES (1956). This appears to be in agreement with the difficulty encountered in producing biologic effects in homo with pig GH. Conclusive evidence concerning the role played by GH in the transport of fat in homo can probably be obtained only in investigations with GH of human origin (BECK *et al.* 1957).

The experiments with LH showed a moderate decrease of the UFA in plasma, which might be compatible with the assumption of an insulin release in agreement with the observations by FOÁ *et al.* (1955) in dogs. The absence of hypoglycemia in experiment 3 and the hyperglycemia in experiment 2 appears, however, to argue against such a mechanism and can for the time being not be explained. But the effect of LH appears closely to resemble that of glucagon. Our results with this hormone are in agreement with the observations of BIERMAN, SCHWARTZ and DOLE (1957) and we agree that the decrease in UFA in plasma induced by glucagon might very well be secondary to the hyperglycemia. Neither the administration of LH nor of glucagon was followed by any change in the concentration of UFA or glycerides that might support the assumption that these hormones have a fat mobilizing effect.

It is evident that the effect, if any, of ACTH on UFA in plasma is much less than that found by GORDON and CHERKES (1956) for adrenaline. The effect of adrenaline may thus not be a consequence of ACTH-release. The dose of ACTH selected (5 I. U./hour intravenously) is probably sufficient to produce maximum stimulation of the adrenals (EIK-NES *et al.* 1955).

The pronounced effect of noradrenaline, which does not produce ACTH-release to the same extent as adrenaline (MADISON 1950), also argues in this direction.

It is known that adrenaline has a much stronger hyperglycemic effect than noradrenaline (DI SALVO *et al.* 1956). The effect on UFA in plasma, on the other hand seems to be of roughly the same order. GORDON and CHERKES (1956) thus found an average increase of UFA from 0.42 meq/l to 0.90 meq/l after 20 minutes intravenous administration of 1 mg adrenaline, which agrees with the results obtained in our experiments with noradrenaline. This does not appear to be quite compatible with the assumption of BIERMAN *et al.* (1957) that the release of UFA is

governed by the availability of carbohydrate. In this conjunction it is of interest to note that the sympathetic nervous system appears to play a considerable role in the mobilization of fat from fat depots (see review by SHAPIRO and WERTHEIMER 1956).

In order to exclude the possibility of the noradrenaline induced increase of UFA in plasma being due to clearing of heparin-induced type we studied a plasma sample taken with citrate 30 minutes after the beginning of the infusion of noradrenaline during experiment 7. To 4 ml of this plasma we added 0.5 ml of human chylous ascitic fluid and the turbidity was followed spectrophotometrically at 500 mμ during 40 minutes incubation at 37° C. No change was observed so the heparin-like mechanism was regarded as unlikely.

With the possible exception of one experiment with noradrenaline, where a small increase of triglycerides was noted, the changes in the glycerides and the phospholipids ran parallel. The changes in these fractions were not greater than what may be expected from the change in the hematocrit on change of posture from the erect position to supine. LANGE (1946) thus showed that such a postural change reduces the hematocrit by 9 to 20 per cent (average 14 per cent) by increasing the plasma volume.

Summary.

1. The immediate effect of intravenous infusion of some hormones on UFA in plasma in human beings was studied on short-term experiments.

2. ACTH and growth hormone of pig origin had no significant effect on UFA.

3. Lactogenic hormone and glucagon had a similar reducing effect on UFA.

4. Noradrenaline increased UFA to roughly the same extent as a corresponding dose of adrenaline.

5. No changes in triglycerides and phospholipids were observed in these experiments.

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Adaptive Structural Changes of the Vascular Walls in Hypertension and their Relation to the Control of the Peripheral Resistance.

By

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In spite of the intense research activity dealing with arterial hypertension, the background of the raised resistance to blood flow is still largely unknown. During the last few decades the main interest has been concentrated on factors which imply a raised activity-level of the vascular smooth muscle cells, such as increased vasoconstrictor fibre activity, larger amounts of bloodborne constrictor agents and increased sensitivity of the effector cells to constrictor influences. Older theories, suggesting that a rigid, sclerotic narrowing of the small resistance vessels should occur, have been rejected, as it has been shown that the vessels of hypertensive subjects generally dilate readily to dilator agents.

Nevertheless it is quite possible that a structural change of the vascular walls, *e. g.* a pure hypertrophy as a response to increased load, once this increased load is established by some "initiating" factor, might significantly affect the haemodynamic characteristics of the resistance vessels in hypertensive disease, without restricting their range of dilator and constrictor responses. Many studies do suggest that a generalized hypertrophic wall thickening of the arterioles takes place in chronic hypertension, more or less independent of its original background (*e. g.* KERNOHAN *et al.* 1929, HEYER and KETTON 1941, FERGUSON and VARCO 1954; see also PICKERING 1955). The possible functional consequences of this type of adaptive structural change, especially as to its effect on the lumen at various levels of smooth muscle tone have, however,

been surprisingly little — if at all — considered, at least when compared with the immense interest devoted to theories implying an increased smooth muscle activity as the background of hypertensive disease. In the present paper an attempt is made to deal with this question by means of both a theoretical and an experimental approach. The problem has previously been briefly outlined in other connection (FOLKOW 1953, 1956).

Part I. Theoretical considerations.

To start with, the characteristics of the resistance vessels will be considered when they are brought to a state of complete smooth muscle relaxation, where their lumina are determined only by the structural characteristics and the distending pressure. This will give the necessary reference point for comparison, as the often unpredictable variable constituted by smooth muscle activity is then no longer an interference. Provided that the distending arterial pressure is at, or above approximately 100 mm Hg, the lumina of maximally dilated vessels seem to behave as rigid tubes, *i. e.* they have reached their maximal diameters (FOLKOW and LÖFVING 1956). For such maximally dilated vessels, which in the course of hypertensive disease have been hypertrophied, the following principal alternatives, as compared with normal thin-walled vessels (N in Fig. 1), are *a priori* possible:

A: The hypertrophic wall change takes place only at the expense of the external diameter, so that the lumen is the same as in a normal vessel at maximal dilatation. (Re increases but Ri unchanged; see Fig. 1).

B: The wall grows predominantly inwardly, so that the lumen also in the maximally dilated state is somewhat decreased, relative to a normal vessel (Re unchanged but Ri decreases).

C: The wall is hypertrophied in such a way that both the external and internal diameters are increased in relation to a normal vessel, when the smooth muscle cells are completely relaxed. (Both Re and Ri increase).

It is hardly possible at present to utilize histological techniques for detecting more subtle changes of this type, and it should be stressed that even small changes can be highly important from a functional point of view. The hazard with histological measurements of small vessels lies in the fact that it can not be known with any certainty whether the vessels observed were completely relaxed

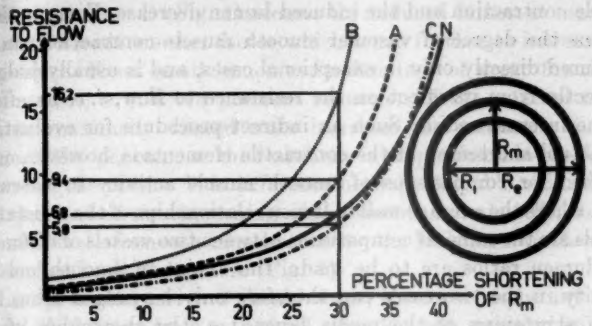

Fig. 1. Effects of different types of a 50 per cent hypertrophic increase of the vascular wall on the relationship between flow-resistance and mean shortening of the media. Its muscle cells are calculated to produce their effect along an imagined circular "main line of force" in the middle of the media, with a radius of R_m. Thickness of wall mass inside this line of contraction is R_m-R_i. This wall mass increases in hypertrophy, but is a constant at different tone levels in a given vessel

N: Normal vessel: $R_i=1$, $R_e=1.2$, $R_m-R_i=0.1$ at maximal dilatation when flow resistance=1.

A: Vessel, where hypertrophy has increased external diameter: $R_i=1$, $R_e=1.3$ $R_m-R_i=0.15$ at maximal dilatation, when flow resistance=1.

B: Vessel where hypertrophy has decreased internal diameter: $R_i=0.9$, $R_e=1.2$, $R_m-R_i=0.15$ at maximal dilatation, when flow resistance=1.5.

C: Vessel, where hypertrophy has increased both internal and external diameter: $R_i=1.1$, $R_e=1.4$, $R_m-R_i=0.15$ at maximal dilatation, when flow resistance=0.7.

Note differences in flow resistance at a 30 per cent mean shortening of the smooth muscle cells.

or to some extent contracted at the moment of tissue fixation, not to mention the influence of the distending pressure at this moment. A smooth muscle shortening will not only reduce the lumen but also increase the wall thickness. For such reasons it is extremely difficult to distinguish between *e. g.* a completely relaxed arteriole with some increase of wall mass and a somewhat bigger arteriole with a normal wall/lumen ratio, but in a state of a slight smooth muscle contraction at the moment of fixation. Therefore, in relation to small blood vessels histological studies are probably only able to reveal more marked hypertrophic changes.

The possibilities would be better for a functional study of the haemodynamic consequences of hypertrophic changes after the pattern of one of the three alternatives A, B and C. Even if, as in alternative A, the maximally dilated resistance vessels are supposed to have a normal lumen diameter, the increased wall mass must in itself entirely change the relationship between a given smooth

muscle contraction and the induced lumen decrease. For practical reasons the degree of vascular smooth muscle contraction can be measured directly only in exceptional cases, and is usually judged indirectly from its effect on the resistance to flow, *i. e.* its effect on the internal radius. Such an indirect procedure for evaluating the actual shortening of the contractile elements is, however, only justified for comparisons of smooth muscle activity in vascular beds, where the average wall to lumen relationships of the resistance vessels are the same. If comparisons between two vessels of different wall/lumen ratios are to be made, the extent of smooth muscle activity in the two cases can therefore only be judged from the mean shortening of the media layer, i. e. the shortening of an imagined "line of force" situated in the middle of the media (Fig. 1). The resulting lumen decrease is now an invalid indicator of the shortening of the contractile elements for the following reasons: — If in one of two vessels the media or (and) the intima is thickened, though both vessels show the same lumen at maximal dilatation, they differ functionally in the important respect that an increased tissue mass is situated within the "line of force" for the smooth muscle contraction in the hypertrophied vessel. At exactly the same activation of the muscle cells — and thus the same shortening of this "line of force" in the two vessels under comparison — the lumen will always be more decreased in the thickwalled vessel, simply due to its increased wall/lumen ratio. The increased tissue-mass, which here is pushed into the lumen by the contraction, will necessarily reduce the lumen more than in the normal vessel (see Fig. 1). For such reasons an increased resistance to flow in chronic hypertensive disease, where a wall hypertrophy of the resistance vessels has developed according to alternative A, does not necessarily mean a proportionally increased smooth muscle activity. Theoretically, the increased wall/lumen ratio must contribute significantly to the increased resistance, to an extent which depends on the magnitude of the adaptive morphological shift. It should then also be stressed that a pure hypertrophic change would still allow a completely normal range of vasodilator and vasoconstrictor responses, as no element of sclerotisation is implied so far. The single, but nevertheless important difference from a thinwalled vessel will be that the hypertrophied vessel will exhibit a higher resistance to flow at a given level of smooth muscle shortening, even if the lumina are the same when the smooth muscle cells are completely relaxed. (Compare "N" and "A" in Fig. 1).

If alternative B (Fig. 1) occurs, the reduced lumen at maximal dilatation adds another factor which tends further to increase the resistance. Even small lumen reductions are of great functional importance, *e. g.* a ten per cent reduction of the internal radius at the maximally dilated state increases the resistance to flow almost fifty per cent, as the resistance is proportional to the fourth power of the radius. This difference between A and B will remain, independent of the shortening of the smooth muscle cells, so that at any level of smooth muscle activity the resistance to flow will be higher in B than in A (Fig. 1).

Lastly, if alternative C should occur, the most important functional difference from a normal vascular bed would then be a *lowered* resistance to flow in the maximally dilated state (see Fig. 1). The vasoconstrictor effects of a given smooth muscle shortening would, however, always be proportionally stronger in C, so that sooner or later curve C will cross curve N (Fig. 1).

Theoretically, it seems unavoidable that the resistance to flow should be affected by an increased wall/lumen ratio after the pattern of one of these alternatives. It might be possible to discover experimentally which of the alternatives for hypertrophic change really occurs in chronic hypertensive disease by comparing the vascular resistance in normal and hypertensive subjects when the vessels are brought to an absolutely maximal dilatation.

Part II. Experimental.

It was considered possible to test experimentally, at least to some extent, the above-mentioned alternatives for the influence of vascular wall hypertrophy on the resistance to blood flow. If it is true, that the hypertrophic change really interferes with the lumen even at complete relaxation of the vascular smooth muscle cells (alternative B), the resistance to blood flow would in a maximally dilated region be somewhat higher in cases of chronic hypertensive disease than in normal individuals. If alternative C should occur, the resistance to flow at maximal dilatation would then be lower than in normotensive individuals. It might be possible to reveal such differences in resistance if a restricted vascular region in normal and hypertensive subjects is brought to a definitely maximal dilatation, while at the same time the blood flow and the perfusion pressure are recorded.

Method.

A group of 34 patients with well-established essential hypertension, but with few or no clinical signs of degenerative vascular lesions, was chosen from a carefully controlled large number of patients suffering from hypertensive disease. Even though a great number of them were under continuous treatment with hypotensive drugs they still had a stable and considerable hypertension of long duration and the majority of them had a definite family history of high blood pressure. Both sexes were represented and the age range extended from 39 to 66 years. Six cases of secondary hypertension, due to known renal damage, were also studied, the age range being 13 to 59 years[1].

As controls, 25 healthy, normotensive subjects, of both sexes and between the ages of 25 and 66 years, were studied. Six of these normal controls were also studied when brought to a state of acute hypertension, induced by a steady intravenous infusion of noradrenaline in amounts sufficient to raise the mean blood pressure about 30 per cent.

The subjects were placed in a supine position with the left forearm at heart level, and measurements of the forearm blood flow were made by means of the venous occlusion plethysmographic method. The left forearm was enclosed in a watertight and very distensible latex tubing, which continued as relatively thick rubber diaphragms at both ends. These diaphragms were attached to the two ends of the plethysmograph, and on the outsides they were supported by adaptable metal iris diaphragms, fastened by screw clamps. The plethysmograph was double-walled and the space between its inner wall and the rubber membrane, enclosing the forearm, was filled with warm water. This was maintained at a temperature of 43° C by means of a hot air stream from an electric fan, which automatically began to circulate heated air between the two wall-layers, as soon as the water bath temperature began to fall below 43°. Great care was always taken to enclose the same fraction of the forearm in the plethysmograph to avoid large variations between the subjects in the amounts of muscle, bone, fat and skin tissue, as various tissue-types probably have different maximal blood flow values. Subjects with obvious increases of subcutaneous fat in their forearms were excluded and in general an attempt was made to make the normotensive and hypertensive subject-groups as equal as possible in age, sex, constitution etc., so that the hypertensive disease constituted the main difference between them.

An ink-writing piston recorder registered the increase of the forearm volume when a venous occlusion pressure of 50 mm Hg was instantaneously applied in a cuff, placed just proximally to the pletysmograph. At each measurement of the forearm blood flow the hand circulation was blocked by a high pressure cuff, placed just distally to the plethysmograph.

[1] The authors are indebted to Dr. B. Hood, Dept of Medicine I, University of Göteborg, for choosing the subjects from his material of hypertensive patients.

Simultaneously with the blood flow measurement the perfusion pressure was recorded from the right arm by means of the Riva Rocci auscultatory method. A general check was made to ensure that the pressure really was the same in the arms, so that the recorded pressure was representative of the inflow pressure to the vessels of the left forearm. As a representative figure of the mean pressure one third of the pulse pressure was added to the diastolic pressure. In a few cases the blood pressure was continuously recorded intraarterially from an in-dwelling polyethylene catheter, introduced percutaneously into the brachial artery. The catheter was connected to a strain-gauge manometer (Elema), operating a sixchannel electrocardiograph (Mingograph, Elema). By damping the pressure oscillations the mean arterial pressure could be directly recorded.

Initially the "resting" blood flow in the forearm was recorded. To induce a maximal dilatation of the vessels of the left forearm it was exposed to increasing periods of ischaemia (two, five, ten, and sometimes fifteen, minutes) together with increasing amounts of work of the forearm muscles during the last minute of ischaemia (twenty to fifty strong hand contractions). In order further to increase the dilator influences on the blood vessels, the periods of total ischaemia were often placed so closely after one another that complete repayment of the blood debt had not occurred in the intervals. Lastly, the high temperature of the water bath, which markedly reddened the skin, was intended to facilitate the dilatation of the skin vessels.

In the subsequent periods of reactive hyperaemia the blood flow was often increased to such an extent, that the "venous reservoir" of the forearm was rapidly filled up, resulting in a considerable elevation of the venous pressure beneath the cuff. In a number of controls the rise of the venous pressure was directly measured by in-dwelling polyethylene catheters introduced in one of the deep forearm veins[1]. This rise naturally lowered the effective perfusion pressure rapidly and this was also obvious from the flattening out of the blood flow curves. To avoid the interference of such rises in venous pressure, it therefore proved necessary to increase the capacity of the forearm venous reservoir to take up the huge blood flow volumes with as little rise of venous pressure as possible. This was accomplished by exposing the part of the forearm which was enclosed in the plethysmograph, to an external pressure of 75—100 mm of mercury, which effectively emptied the forearm veins, if the hand circulation had been obstructed so that no extra blood was trapped in the hand veins. It then proved possible to record the huge blood flows with only insignificant rises in venous pressure during the first 4 to 5 seconds, long enough to allow a fairly correct estimation of the flow from the recorded curve. Therefore, when the venous vessels had been pre-emptied, an extra blood amount corresponding to about 3—4 per cent of the tissue volume could be accumulated in the vascular bed with only a small rise in venous pressure, which was controlled by a series of direct venous pressure measurements. Otherwise,

[1] The authors are indebted to Dr. O. Celander for valuable help with these direct measurements of the venous pressure changes.

only half this blood volume can be accumulated in a forearm without disturbing rises of the venous pressure (GREENFIELD and PATTERSON 1954).

Results.

Fig. 2 shows a series of representative plethysmographic recordings of the forearm blood flow immediately after varying periods of ischaemia in a normotensive subject, before and during noradrenaline infusion, and also in two subjects with respectively renal and essential hypertensive disease.

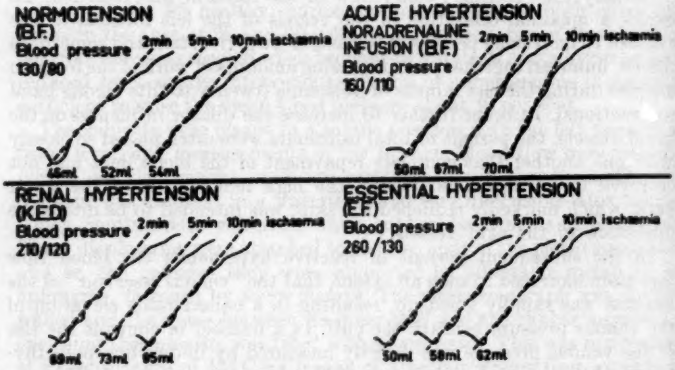

Fig. 2. Plethysmographic recordings of forearm blood flow when attempts are made to induce a maximal dilatation. Upper left: Normotensive subject. Upper right: Same subject, made acutely hypertensive by noradrenaline infusion. Below two cases with chronic hypertension.

Fig. 3 illustrates the correlation between the duration of the ischaemic period (which can be said to be related to the local concentration of dilator factors), and the mean blood flow values immediately after a period of ischaemia. The curves obtained can in fact be looked upon as a type of dose-response correlations, relating the concentration of dilator agents to their inhibitory effects on the vascular smooth muscle cells. To judge from the characteristics of the curves, the vessels dilate promptly both in normotensive and hypertensive subjects and an almost maximal dilatation seems to be reached after only five minutes of ischaemia in both groups. This makes it probable — though it constitutes no definite proof — that a complete or nearly complete relaxation

of the vascular smooth muscle cells really has been induced by the procedures used in most of the cases studied.

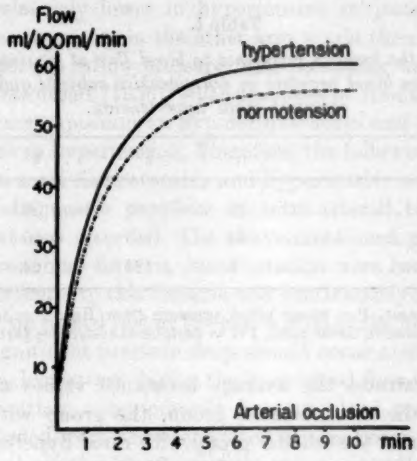

Fig. 3. The approximate correlation between the duration of ischeamia and the forearm blood flow values in normotensive and hypertensive subjects.

The ratio of the mean arterial blood pressure and the maximally obtainable blood flow, calculated per 100 ml of forearm-tissue per minute, represents the flow resistance left when all smooth muscle activity is eliminated. Under these circumstances only the structural characteristics of the resistance vessels and the pressure should affect the haemodynamics. When the mean pressure is *e. g.* 100 mm mercury and the blood flow 50 ml per 100 ml of tissue per minute, the resistance is set at 2 units, etc.

In five normotensive subjects the resistance to flow at complete relaxation of the vascular smooth muscles was measured before and during an acute hypertension was induced by infusion of noradrenaline, and was found to be essentially the same at the two perfusion pressure levels. This should also be the case when the raised resistance is only due to increased smooth muscle activity with no change in vascular wall structure, and if the maximally dilated resistance vessels are very little distensible at these ranges of transmural pressures. To judge from experiments on cats, maxi-

mally dilated resistance vessels seem to have reached an "indistensible jacket" at mean arterial pressures of 100 mm Hg and higher (FOLKOW and LÖFVING 1956).

Table I.

Mean values of the forearm resistance to blood flow at maximal dilatation as related to mean blood pressure in normotensive subjects and in cases of essential renal hypertension.

	n	P	F	PR
Normotension	25	96	53	1.81
Ess. hypertension	34	149	60	2.48
Renal hypertension	6	134	64	2.09

n = number of cases, P = mean blood pressure (mm Hg), F = forearm blood flow (ml/100 ml forearm tissue/min), PR = peripheral resistance (arbitrary units).

Table I illustrates the average resistance values at maximal dilatation in the normotensive group, the group with essential hypertension, and the smaller group with renal hypertension. The results indicate that at maximal dilatation, also, there seems to be a higher resistance to flow in subjects with essential hypertension as compared with normotensive subjects and statistically this difference is significant ($p < 0.01 \text{—} > 0.001$), provided it could be ascertained that the vessels really were maximally dilated. Although this is very probable as mentioned above (Fig. 3), this can for obvious reasons not be proved beyond doubt in experiments of this type on human subjects. For these reasons we prefer to look upon the results as suggestive evidence rather than any experimental proof of a change according to alternative B. In any case, if it is assumed that in these cases with a well-established essential hypertension a certain wall hypertrophy has taken place, the results make it highly unlikely that this hypertrophy should have occurred after the pattern of alternative C ($p = < 0.001$). The cases with definitely renal hypertension are closer to the normotensive ones, but they are too few to allow any definite conclusions.

It was, however, necessary also to exclude the possibility that the somewhat higher vascular resistance, which seemed to exist also at maximal dilatation in hypertensive subjects, as compared with normotensive individuals, was not simply due to a narrowing of the bigger arteries to the forearm. They are not brought to a maximal dilatation by the procedures used and their relative

importance for the total flow resistance must be somewhat increased when the small "resistance vessels" are maximally dilated. If this is the case, the true inflow pressure to the vasodilated forearm might be relatively lower in hypertensive subjects, so that the pressure measurements in the other arm would then be unvalid as a standard for the inflow pressure. This possibility was pointed out to us by DORNHORST (1956), when the present results were briefly outlined at a symposium on hypotensive drugs and the control of vascular tone in hypertension. Therefore, the following comparison was made in some normotensive and hypertensive subjects, where for clinical-diagnostic purposes an intra-arterial blood pressure measurement was recorded. The above-mentioned procedures for inducing a maximal forearm vasodilatation were here used, while the inflow pressure to this forearm was continuously recorded with the catheter tip placed just at the elbow level of the brachial artery. If a more significant pressure drop should occur along the subclavian and brachial artery during the huge blood flow at the peak of the vasodilatation, a temporarily lowered blood pressure should then be recorded from the vasodilated arm as compared with the other arm, where the blood pressure was repeatedly checked by the Riva Rocci auscultatory method. No more important difference was, however, observed either in normotensive or hypertensive cases, at least not during the first 5—6 seconds of reactive hyperaemia, during which period the blood flow recordings were always performed. Further, the small pressure falls, that sometimes were noted, were not bigger in the hypertensive cases as compared with normotensive ones. Thus no evidence was obtained indicating that a significantly bigger pressure drop should occur along the bigger arteries in the hypertensive subjects as compared with the normotensive ones.

General discussion.

In the older literature a sclerotic narrowing of the resistance vessels was sometimes discussed, a hypertrophic wall thickening has often been described, and more recently a possible narrowing due to "water-logging" of their walls has been suggested (TOBIAN and BINION 1952), but the detailed dynamic consequences of changes in the wall/lumen ratio have practically not at all been analyzed earlier. The present considerations, which previously have been partly outlined in other connections (FOLKOW 1953,

1956), are an attempt to analyze the changed functional characteristics with its haemodynamic consequences, which will appear once the wall/lumen ratio of the resistance blood vessels is increased. Increases of wall thickness are said to take place more or less uniformly as a result of what seems to be a simple hypertrophic growth, predominantly of the media layer, in chronic hypertension (for lit. see *e. g.* PICKERING 1955). It also seems to occur within the pulmonary vascular bed when the blood pressure in the lesser circulation is increased (FERGUSON and VARCO 1954), and hypertrophy is in fact a generally occurring reaction in most tissues, when they are exposed to increased strain. For the reasons stated in Part I, it seems very probable that an increase of wall thickness in relation to a given vascular lumen, by its very existence, must potentiate the lumen reduction caused by a given smooth muscle shortening. If, however, one shall be able to judge whether such a structural-physical factor will exert any more dominant influence in chronic hypertensive disease, it is necessary to know how big this "given" lumen is in hypertension and normotension under circumstances when all smooth muscle activity and its dynamic influence on the lumen is abolished. The lumina of the maximally dilated resistance vessels thus create the necessary "baseline" for an evaluation of the haemodynamic consequences of a hypertrophic wall change. As discussed in Part I it is a *priori* possible that the vascular lumen at maximal dilatation remains unchanged as compared with the state before the hypertrophic process started (alt. A, Fig. 1), that it has decreased (alt. B) or increased (alt. C). The experiments described in Part II may here give some information, though, as mentioned, they should be looked upon more as suggestive evidence than a definite experimental proof. Provided that the majority of the hypertensive subjects studied really had established a certain hypertrophic vascular change, which there are good reasons for believing was the case to judge from earlier studies (see PICKERING 1955), the experimental results indicate that it is very unlikely that hypertrophy should have occurred according to alternative C. In other words, there is no experimental evidence to indicate that the lumina of the maximally dilated resistance vessels should have increased in the process of hypertrophic vascular change, which is the haemodynamically important point in this connection. This is in agreement with results obtained in earlier studies of forearm vasodilator responses in hypertensive and normotensive subjects (PICKERING 1936, PRINZMETAL and

WILSON 1936, EICHNA and WILKINS 1941) though, to judge from the highest flow values recorded in these investigations, maximal dilatations were probably not always obtained in the cases studied. If anything, the present experiments suggest that the lumina of the maximally dilated vessels on an average are somewhat decreased, at least in essential hypertensive cases as compared with normotensive individuals, indicating a hypertrophic change after the pattern of alternative B. For reasons discussed previously, however, it can not be definitely excluded that the lumina at maximal dilatation in fact remain fairly unchanged (alternative A). In any case, *even if in chronic hypertension the vascular lumina at maximal dilatation should be the same as in normotensive cases, the increased tissue mass inside the line of force for smooth muscle contraction will by its very existence always create a higher resistance to flow at any given activity level of the smooth muscle cells.* Thus, as was briefly outlined in an earlier communication (FOLKOW 1956), and more recently discussed also by CONWAY (1958) and REDLEAF and TOBIAN (1958), the hypertrophic wall change can be said to imply "a potentiation" of the luminal decrease caused by vascular smooth muscle contraction. If it is so that the lumen also at maximal dilatation is decreased, this will further tend to increase the resistance at all levels of vascular tone.

These considerations open up the possibility that the raised resistance to flow, at least in well-established essential hypertension, is not only a consequence of increased smooth muscle activity, though such a functional change is undoubtedly indispensible as a "trigger mechanism" especially important in many types of secondary hypertension. It is not impossible, however, that in essential hypertension the trigger mechanism may be constituted simply by intermittent, moderate increases of vasoconstrictor fibre activity, as a response to *e. g.* "normal" stress situations in daily life, in the same way as some individuals then react with intermittent vagal hyperactivity and consequent gastric hypersecretion. Even if under such circumstances the blood pressure for years may remain at fairly normal levels under resting conditions, it is obvious that intermittent rises in blood pressure constitute an increased average pressure load for the resistance vessels, which may be enough to initiate gradually a secondary vascular wall hypertrophy in some cases. It is here possible that great individual differences exist with regard to the tendency of hypertrophic adaptation of tissues which are exposed to increased strain. It is

often claimed that individuals with essential hypertension generally have a mesomorphic constitution and that the disease seems to exhibit a definite element of heredity, and for such reasons it has been suggested that their increased vascular resistance might simply be a local manifestation of generalized excessive mesenchymal endowment (BACKER 1956). Is it possibly so that these individuals obtain a well-established hypertension mainly because their tissues, in this particular case their resistance blood vessels, show an inherent trend towards hypertrophic changes, which become manifest even at intermittent pressure loads so moderate, that they hardly can be said to exceed the normal range of blood pressure variations? If this is so, the borderline between normotension and essential hypertension is indeed a fleeting one, as has also been suggested by PICKERING (1955), setting out from different aspects. Whatever the case, once a hypertrophic vascular wall change is there, it will exert its "potentiating" effect on the vascular responses to the smooth muscle reactions, the more so the more pronounced it is. This means that the vascular bed in chronic hypertensive disease slowly becomes structurally more or less adapted to maintain its haemodynamic equilibrium at a higher resistance and pressure level, though this in no way means that its range of vasodilator and vasoconstrictor responses becomes reduced. If the wall thickening is more marked, little or no increase of smooth muscle activity should be needed to maintain a considerably elevated flow resistance, though especially in cases of secondary hypertension increased tone is probably always the dominating feature.

A gradual hypertrophic change in the course of an originally functional disturbance, whether created by more or less intermittent increases of vasoconstrictor fibre activity or by humoral agents, may be responsible for the often noted "stabilization" of the hypertensive condition. Furthermore, the interaction between a more or less intermittent, purely functional component, and a secondary wall/lumen increase, might constitute the introduction of a vicious circle with a steadily climbing pressure level, which in its turn seems to have a tendency to induce true vascular lesions. The renal vessels are especially susceptible to such lesions, with possibility of a more general engagement of the renin-angiotensin mechanism.

Other possible secondary mechanisms, more acute in appearance but also *per se* unrelated to specific vasoconstrictor influences,

should also be briefly discussed in this connection. It is known, for instance, that the resistance to blood flow within the kidney promptly increases when the pressure is raised. It has recently been suggested that this "autoregulation" of the renal blood flow is one of the haemodynamic consequences of the interesting concept of a partial separation of cells and plasma within the renal vascular bed (PAPPENHEIMER and KINTER 1956). To some extent it might also be due to a local vasoconstrictor response to the increased pressure as such (see *e. g.* MILES *et al.* 1954). Also the resistance of the cerebral vessels tends to increase when the blood pressure is raised (see *e. g.* FOG 1934, 1937, KETY 1956, and others). Similar reactions can in fact also be observed in other vascular regions, *e. g.* in the skeletal muscles (FOLKOW 1949). This locally induced, "intrinsic" change of vascular tone is, to some extent at least, due to the mechanical stimulus constituted by the pressure as such, which seems to facilitate the automaticity of the smooth muscles in the small vessels (BAYLISS 1902, FOLKOW 1949, HILTON 1953, WOOD *et al.* 1955, GOLENHOFEN and HILDEBRANDT 1957, WIEDEMAN 1957 and others). Probably quantitatively more important, however, is the local chemical change which occurs when blood flow increases as a result of the increased pressure head (see FOLKOW 1952, FOLKOW and LÖFVING 1956). The raised blood flow will cause a lowering of the local concentration of vasodilator factors, steadily produced by the tissue, and the tissue oxygen tension will increase. Other factors being unchanged, both these shifts of the environment of the vascular smooth muscle cells tend to increase their tone. Therefore, the flow does not increase in proportion to the pressure, as the resistance becomes raised simply because of a somewhat diminished local concentration of normal vasodilator factors, which tends to increase vascular tone. If the interference of compensatory shifts in vasoconstrictor fibre activity is eliminated, the influence of mechanisms of this character can be observed also when the total systemic vascular bed of an animal is perfused with normal blood from a donor at different pressures, provided that the blood is not traumatized in pumps, etc. which releases factors that depress vascular tone (FOLKOW 1952 a, b,). If the nervous adjustments are not eliminated in acute rises of blood pressure, they will of course by means of the baroreceptor reflexes effectively mask the local reactions of the vessels to pressure increases, but in chronic hypertension this counteracting nervous influence to other factors, which have increased vascular

tone, seems to be largely absent, as the baroceptors then behave as if they were "reset" to the higher pressure level (see *e. g.* McCUBBIN *et al.* 1956).

Obviously the above-mentioned acute mechanisms tend to increase the resistance somewhat in many haemodynamically important vascular regions, once a primary blood pressure increase is established, and their possible engagement in the specific hypertensive state seen in aortic coarctation has recently been discussed (PATTERSON *et al.* 1957). In general it must be realized that a number of secondary mechanisms both acute and more slowly-evident, whose nature is basically different from the more specific vasoconstrictor influence of the primary mechanism, may be of considerable importance in the creation and the maintenance of a hypertensive state.

It is fully realized that an experimental analysis of the qualitative and quantitative interrelations of possible factors, engaged in raising the peripheral resistance in the various types of chronic hypertensive disease, meets with overwhelming difficulties and many hazards, which a study of the actual literature also clearly bears out. Intense interest has in latter years been directed towards factors that may increase smooth muscle activity, or increase their sensitivity. This has proved to be very justifiable, specially in the different types of secondary hypertension, but it also seems to have led to the result that other haemodynamically important mechanisms have been more or less forgotten. Especially the attempts to explain so-called essential hypertension as only caused by increased smooth muscle activity seem to have only limited success. Therefore the experiments and deductions herein presented are mainly intended to direct interest also to some entirely different factors of possible importance in chronic hypertensive disease.

Summary.

1. The functional importance of an increased wall/lumen ratio due to *e. g.* hypertrophy of the walls of the resistance vessels in hypertensive disease has been dealt with. It is theoretically highly probable that the mere existence of an increased wall mass must cause a proportionally bigger lumen decrease for a given smooth muscle shortening, so that a structurally based "potentiation" of the vasoconstrictor response is obtained.

2. For this reason a given level of smooth muscle tone will probably result in a higher flow resistance in hypertensive disease, where vascular wall hypertrophy has taken place, as compared with the normal vascular bed, provided that the maximally dilated vessels do not show an increase of the lumen as a result of the hypertrophic change. Experimental evidence suggests that this is not the case; if anything the maximally dilated resistance vessels seem to be slightly narrowed in cases of well-established essential hypertension.

3. In addition it is pointed out that a primary increase of pressure may in many haemodynamically important regions raise the vascular tone, simply due to some strictly local influences on the resistance vessels, where no engagement of specific vasoconstrictor agents or increased smooth muscle sensitivity is implied.

4. Such more chronic and acute secondary consequences of a primary rise of blood pressure may importantly contribute to the increased flow resistance, and possibly most so in so-called essential hypertension.

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A Method for the Fluorimetric Determination of Adrenaline and Noradrenaline in Tissues.[1]

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Only fluorimetric methods appear to be sensitive enough for the chemical determination of adrenaline and noradrenaline in tissues in general and in body fluids. Of the two fluorimetric methods available, *i. e.*, the THI (trihydroxyindole) method (EHRLÉN 1948, LUND 1949, 1950, EULER and FLODING 1955) and the ethylene diamine condensation method (NATELSON, LUGOVOY and PINCUS 1949, WEIL-MALHERBE and BONE 1952, 1957), the former is more convenient to use and appears to be superior as regards specificity (EULER 1956, VALK and PRICE 1956). In this method adrenaline and noradrenaline are oxidized to adrenochrome and noradrenochrome, respectively. These compounds are then rearranged in alkali to strongly fluorescent trihydroxyindoles: adrenolutine and noradrenolutine, respectively. Differential estimation of these compounds can be performed by utilizing differences in oxidation rates or, more conveniently, in spectral characteristics.

The fluorimetric estimation of catechol amines in tissues and body fluids requires an extraction and purification procedure in

[1] The method has been briefly described in earlier reports (BERTLER, CARLSSON and ROSENGREN 1956, CARLSSON *et al.* 1957).

[2] With technical assistance by Miss Carin Larsson.

order to remove interfering substances. Such procedures are complicated by the instability of catechol amines in neutral and alkaline solutions and by their slight solubility in many organic solvents. Extraction with acid ethanol or trichloracetic acid (which is then removed by ether extraction), adsorption on alumina and subsequent elution with acetic acid has been the standard procedure in connection with bioassay techniques and has proved useful also in connection with fluorimetric methods (see EULER 1956 and references, EULER and FLODING 1955, PRICE and PRICE 1957, COHEN and GOLDENBERG 1957). A weak cation exchange resin, Amberlite IRC-50, has been employed for the isolation of catechol amines from adrenal extracts and may prove useful also for other tissues (BERGSTRÖM and HANSSON 1951, KIRSHNER and GOODALL 1957). In the method described below we have chosen a strong cation exchange resin (Dowex 50), which seems to be more convenient to use than Amberlite IRC-50. The recovery figures obtained with Dowex 50 appear to compare favourably with those obtained with other adsorbants.

Preparation of Extracts.

The animals (rabbits) are killed by air-embolism. The brain, heart and adrenals are immediately dissected out. The organs are placed in beakers containing a few ml 0.4 N perchloric acid. They are then homogenized by means of an "Ultra-Turrax" homogenizer. To the homogenates 0.4 N perchloric acid is added up to 30 ml per brain and heart and 10 ml per pair of adrenals. The homogenates are allowed to stand for 30 minutes and are then centrifuged in a refrigerated centrifuge for 10 minutes at about 9,000 *g*. The supernatants are filtered and the residues of the brain and heart are re-extracted twice with 30 ml 0.4 N perchloric acid. In some experiments a third re-extraction has been performed. No catechol amines could be detected in this portion (Table I). For the adrenals re-extraction once with 10 ml perchloric acid solution is sufficient. All the steps of extraction are carried out at about 0° C.

The combined filtrates are placed in a freezing box overnight (— 19° C). After thawing, the pH of the extracts is adjusted to about 4 with 5 N potassium carbonate using an automatic titrator or brom phenol blue (one drop of a 0.04 per cent solution in ethanol) as an indicator. The potassium carbonate solution should be added drop-wise and under stirring. The potassium perchlorate is spun

Table I.

The adrenaline + noradrenaline content of four subsequently prepared extracts of heart and brain.

The calculated values were obtained on the assumption that the catechol amines are evenly distributed in the water phase.

The figures are given as per cent of total.

		Extraction no. I	II	III	IV
Brain 1[1]	Found	80	18	2	0
	Calculated ...	82	15	3	0
Brain 2	Found	78	18	4	0
	Calculated ...	78	17	4	1
Heart 1	Found	84	13	3	0
	Calculated ...	84	14	2	0
Heart 2	Found	87	11	2	0
	Calculated ...	88	11	1	0

[1] Example. Brain 1 weighed 8.85 g and would thus contain 6.6 ml water. The total volume of water in the homogenate was thus 30 + 6.6 = 36.6 ml. The volume of the supernatant obtained after extraction I was 30 ml or 82 per cent of the total volume of water in the homogenate. This supernatant was found to contain 80 per cent of the total amount of catechol amines recovered from all the four supernatants.

down at 0° C. The precipitates are washed with a few ml of glass-distilled water, which is then added to the extract.

Comments: Perchloric acid solution is a suitable extraction fluid for several reasons. Potassium perchlorate is only slightly soluble in cold water (7.5 g per 1,000 ml water). The acid can be neutralized with potassium carbonate or potassium hydroxide. The bulk of perchlorate is thus precipitated. The break through capacity of the resin will then be but slightly reduced. Potassium carbonate is preferable to potassium hydroxide as the risk of oxidation of the catechol amines is smaller. Acid ethanol and trichloracetic acid have also been tried. The use of the former as extraction fluid necessitates that the columns be carefully freed from water. Otherwise the lipids of the extract will emulsify at the dilution of ethanol with water. At the same time air bubbles may appear in the column. The trichloracetic acid can be removed by extraction with diethyl ether, but according to our experience this procedure

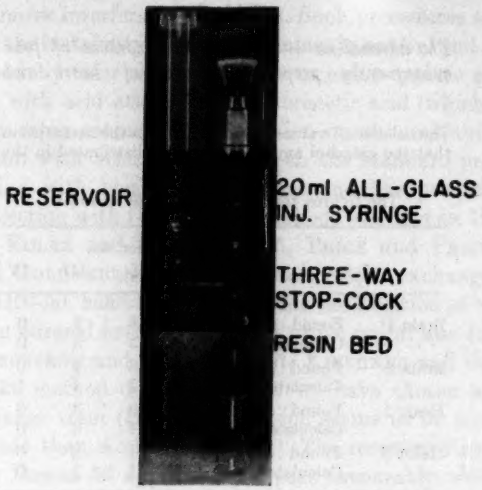

Fig. 1. Apparatus for the ion-exchange procedure.

seems to require special precautions in order to prevent appreciable loss of catechol amines.

In order to preserve the columns it is essential to obtain clear extracts. In fresh extracts a fine suspension may be present, which may be difficult to remove. It was observed that if the extracts were left in the freezing box overnight this suspension could be easily spun down (together with the potassium perchlorate) after thawing and neutralization.

No destruction of catechol amines in the acid extract can be detected after a week, which is the longest time investigated.

Some experiments have been performed with a *simplified extraction procedure*, in which the organs are extracted with only one portion of perchloric acid solution. After centrifugation of the homogenate a measured volume of the supernatant is taken for assay. The total amount of catechol amines in the homogenate is calculated by multiplying the catechol amine content of the examined part of the extract by the factor:

$$\frac{\text{water content of tissue} + \text{extraction volume used}}{\text{volume taken for assay}}$$

Table II.

Uptake of noradrenaline (NA), adrenaline (A), dopamine (DA), and 3,4-Dihydroxyphenylalanine (DOPA) by a Dowex 50 column (200 mg) from a solution containing potassium perchlorate and potassium chloride in about the same amounts as in the brain extract.

Elution of the amines was performed by means of N HCl.

Added	Resin form	Found, μg		
		in effluent of the model extract	in eluate 0—8 ml	in eluate 8—16 ml
NA, 20 μg	Na^+	0.6	19.7	0.0
A, 20 μg	»	0.3	18.8	0.0
DA, 20 μg	»	0.3	11.9	5.3
DOPA, 20 μg	»	19.9	0.3	0.0
DOPA, 20 μg[1]	H^+	2.1	16.3	0.0

[1] The pH of the solution put on the column was 2.5 instead of the usual pH 4.

Experiments were performed to check this procedure. The brains and hearts from two rabbits were extracted four times with 30 ml 0.4 N perchloric acid. The supernatant from each of the four extracts was measured and examined for its catechol amine content. The values thus obtained were compared with the values calculated on the assumptions a) that the water content of the tissues investigated is 75 per cent and b) that the catechol amines are present only in the water phase of the residue, the concentration being the same as in the supernatant. As seen in Table I there was a close agreement between observed and calculated values.

Purification of Extracts.

A Dowex 50 column is used (200 to 400 mesh, dry weight 200 mg, dimensions 20 $mm^2 \times 12$ mm). Before the resin is taken into use it is washed several times with 2 N hydrochloric acid. The column is ready for use after the solutions mentioned below have been passed through it.

a) 20 ml 2 N hydrochloric acid
b) 5 ml glass-distilled water
c) 10 ml 1 N sodium acetate — acetic acid buffer, pH 6.0.
d) 5 ml glass-distilled water.

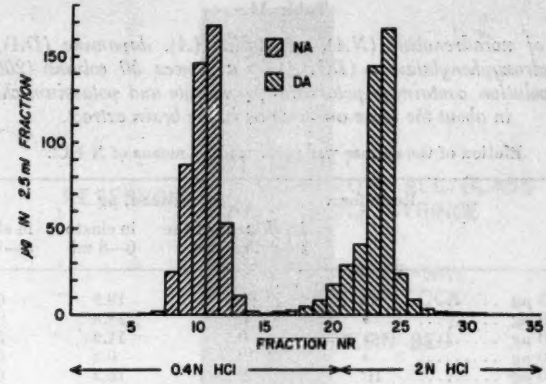

Fig. 2. Separation of noradrenaline (NA) and dopamine (DA) by ion-exchange chromatography.

Column: Dowex 50, Na^+, 20 $mm^3 \times 90$ mm. Elutriant 0.4 and 2.0 N HCl.

An aliqout of the extract is passed through the column. After rinsing with 20 ml glass-distilled water, elution is performed with 8 ml N hydrochloric acid. The flow rate should be kept at 0.25 ml per minute. In order to pass the solutions through the column at this rate pressure must be applied. For this purpose the apparatus illustrated in Figure 1 has been constructed. The rate of flow can be controlled by varying the load on the top of the syringe.

The extract of suprarenal medulla was found to be pure enough to permit fluorimetric determination directly with the procedure described below.

Comments: The uptake by the columns of noradrenaline and adrenaline and the elution of the amines from the resin have been studied in experiments with pure solutions. In Table II a few such data are given. Some results of experiments with dopamine and DOPA (3,4-dihydroxy-phenylalanine) have been included in the table. Twenty μg of each catechol derivative was added to 45 ml 0.4 N perchloric acid containing potassium chloride to an amount roughly corresponding to the salt content of half the brain. This model extract was treated in the same way as the heart and brain extracts. It was neutralized to pH 4 and passed through a column at 0.25 ml per minute, the effluent being collected in 15 ml portions. Elution was performed with 2 portions

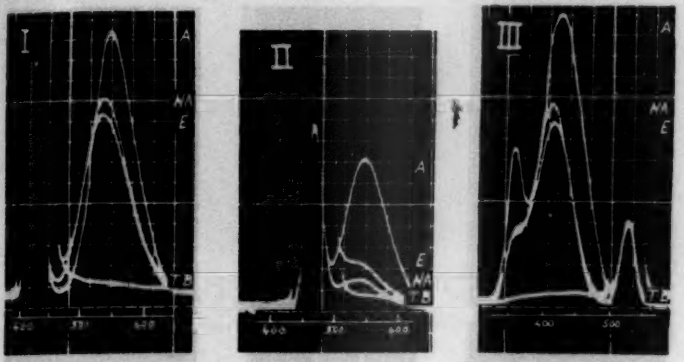

Fig. 3. Activation and fluorescence spectra of an eluate from a normal rabbit's heart after oxidation and rearrangement in alkali.

I. Fluorescence spectrum. Activating wave-length 410 mμ.
II. Fluorescence spectrum. Activating wave-length 455 mμ.
III. Activation spectrum. Fluorescent wave-length 540 mμ. A: Adrenaline standard, 0.085 μg per ml. NA: Noradrenaline standard, 0.085 μg per ml. E: Eluate from heart. TB: Tissue blank.
The scale indicates wave-length in mμ.
***Note.* The peaks to the left in I and II and to the right in III are due to light scattering.**
The activation and fluorescence peaks obtained from adrenaline are 425 and 545 mμ, respectively. The corresponding peaks for noradrenaline are at 410 and 535 mμ, respectively. The spectra of the eluate come close to those obtained from noradrenaline.

of 8 ml N hydrochloric acid. It was thus observed that adrenaline, noradrenaline and dopamine are quantitatively taken up from the solutions both by the hydrogen and the sodium form of the resin. DOPA passes freely through the sodium form of the resin, but most of it is retained by the hydrogen form. Whereas noradrenaline and adrenaline are quantitatively eluted by 8 ml N hydrochloric acid, dopamine is held more strongly by the resin, and a considerable amount of it appears in the following 8 ml. Under appropriate conditions dopamine can be separated from the other two catechol amines. Thus with the column used reasonably good separations and recoveries can be obtained by eluting first with 6 ml N HCl and then with 6 ml 2 N HCl. A few experiments have been performed with a larger column. Fig. 2 illustrates the complete separation of dopamine from noradrenaline on such a column (Dowex 50, 200 to 400 mesh, dry weight 1 g, 20 mm^2 × 90 mm; Na^+ form). Five hundred μg of each of the amines

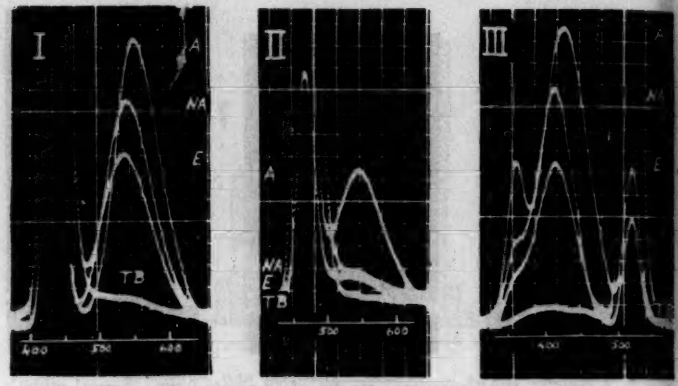

Fig. 4. Activation and fluorescence spectra of an eluate from a normal rabbit's brain after oxidation and rearrangement in alkali.

I. Fluorescence spectrum. Activating wave-length 410 mμ.
II. Fluorescence spectrum. Activating wave-length 455 mμ.
III. Activation spectrum. Fluorescent wave-length 540 mμ. A: Adrenaline standard, 0.035 μg per ml. NA: Noradrenaline standard, 0.065 μg per ml. E: Eluate from brain. TB: Tissue blank
The scale indicates wave-length in mμ.
Note. The peaks to the left in I and II and to the right in III are due to light scattering.
The spectra of the eluate come close to those obtained from noradrenaline. For further explanation, see Fig. 3.

in 2 ml 0.01 N hydrochloric acid were placed on the top of the column, followed by 2 ml water, 50 ml 0.4 N and 37.5 ml 2 N hydrochloric acid. The effluent was collected in 2.5 ml portions which were examined for catechol derivatives using the THI method (see below) and the ethylene-diamine reaction of WEIL-MALHERBE and BONE (1952) as modified by KÄGI, BURGER and GIGER (1957).

In the present experiments a batch of Dowex 50 was used, which was not defined with regard to the degree of cross linking. It has later been observed that the behaviour of the resin used comes close to that of Dowex 50, X-8. It has also been found that Dowex 50, X-4, is perhaps preferable for the present purpose. Here the dimensions of the column and the volumes of the eluates should be somewhat different from those given in the present paper. Details concerning the use of Dowex 50, X-4, will be reported in a future communication.

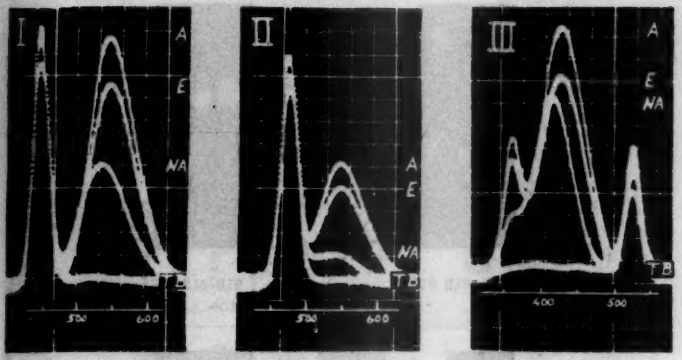

Fig. 5. Activation and fluorescence spectra of an extract from a normal rabbit's adrenals after oxidation and rearrangement in alkali.

I. Fluorescence spectrum. Activating wave-length 425 mμ.
II. Fluorescence spectrum. Activating wave-length 455 mμ.
III. Activation spectrum. Fluorescent wave-length 540 mμ. A: Adrenaline standard, 0.080 μg per ml. NA: Noradrenaline standard, 0.080 μg per ml. E: Extract from adrenals. TB: Tissue blank.
The scale indicates wave-length in mμ.
Note. The peaks to the left in I and II and to the right in III are due to light scattering.
The spectra of the extract come close to those obtained from adrenaline. For further explanation, see Fig. 3.

Assay of Catechol Amines.

The adrenaline and noradrenaline in the eluates are determined fluorimetrically after oxidation and subsequent rearrangement to adrenolutine and noradrenolutine, respectively, essentially according to EULER and FLODING (1955). After adjustment of pH to about 6 by means of 5 N potassium carbonate, using brom thymol blue (one drop of a 0.04 per cent solution in ethanol) as an indicator or an automatic titrator, an aliqout of 1 to 2 ml is taken for assay. One ml 0.1 M phosphate buffer, pH 6.5, 0.1 ml zinc sulfate solution (0.25 g $ZnSO_4$, 7 H_2O in 100 ml water) and glass-distilled water to give a total volume of 8.9 ml are added. Oxidation is performed by addition of 0.1 ml 0.25 per cent potassium ferricyanide. After 2 minutes 1.0 ml of a mixture of 9 parts of 5 N sodium hydroxide and 1 part of a 2 per cent solution of ascorbic acid is added. After 5 to 30 minutes the samples are read in an Aminco-Bowman spectrophotofluorometer. Using this in-

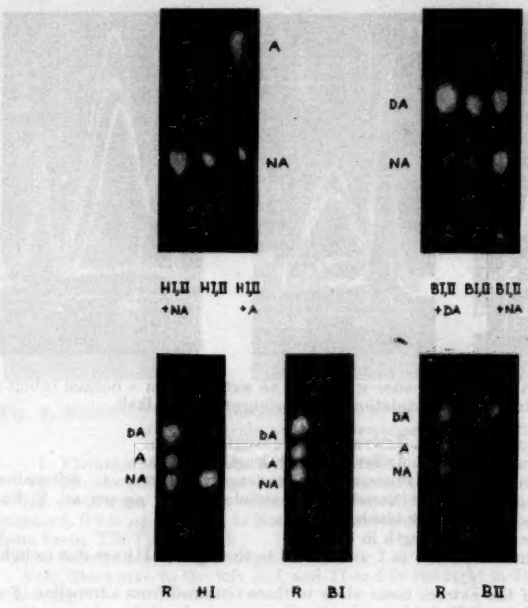

Fig. 6. Paper chromatography of heart and brain eluates. Upper chromatograms: phenol-HCl. Lower chromatograms: butanol-HCl. H I: Heart, first eluate (0—8 ml N HCl). H II: Heart, second eluate (8—16 ml N HCl). H I, II: Heart, first and second eluates combined. B I, B II, and B I, II: the corresponding eluates of brain. R: References. DA: dopamine. A: adrenaline. NA: noradrenaline.

Each eluate corresponds to two-thirds of a rabbit's heart or brain, except for the butanol chromatogram of B II, which corresponds to two brains.

strument the activation peaks of adrenolutine and noradrenolutine are at 425 and 410 mμ, respectively, the corresponding fluorescence peaks being at 545 and 535 mμ, respectively. (All wave-lengths given in the present paper are uncorrected instrumental values.) The activation spectra of adrenolutine and noradrenolutine are different enough (Fig. 3—5) to allow a differential estimation of the two amines by measuring the fluorescence intensity at different activating wave-lengths (*e. g.* 410 and 455 mμ). Readings are performed at a fluorescent wave-length of 540 mμ. The adrenaline and noradrenaline in the sample can then be calculated.

Table III.

Example of analysis of adrenaline (A) and noradrenaline (NA) in a rabbit's heart.

	1 Sample	2 Tissue blank	3 A, intern. stand.	4 NA, intern. stand.	5 A, stand.	6 NA, stand.	7 Reagent blank
NA[1], ml	—	—	—	1.0	—	2.0	—
A[1], ml	—	—	1.0	—	2.0	—	—
Eluate, ml	2.0	2.0	2.0	2.0	—	—	—
Buffer, ml	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$ZnSO_4$, ml	0.1	0.1	0.1	0.1	0.1	0.1	0.1
H_2O, ml	5.8	5.9	4.8	4.8	5.8	5.8	7.8
$K_4Fe(CN)_6$, ml	0.1	—	0.1	0.1	0.1	0.1	0.1
NaOH-asc. acid, ml	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Fluorescence intensity at fluor. w.-l. 540 mμ, arbitrary units.							
Activ. w.-l. 410 mμ	34	2.0	113	108	80[2]	65	0.6
Activ. w.-l. 455 mμ	20.5	2.8	61	28.0	80[2]	14.0	1.0

[1] Containing 0.5 μg catechol amine base per ml.
[2] The adrenaline standard was set to 80.

Table IV.

Differential estimation of adrenaline (A) and noradrenaline (NA) in pure solutions.

Activating wave-lengths 410 and 455 mμ.
Fluorescent wave-length 540 mμ.

Added to sample		Found in sample	
μg			
A	NA	A	NA
0.05	0.95	0.06	0.94
0.10	0.90	0.10	0.92
0.25	0.75	0.21	0.77
0.50	0.50	0.55	0.44
0.50	0.50	0.54	0.48
0.50	0.50	0.52	0.50
0.75	0.25	0.74	0.25
0.90	0.10	0.90	0.06
0.95	0.05	0.94	0.06

Blanks ("tissue blanks") are treated in the same way as the samples except that no potassium ferricyanide is added. Decomposed (or "faded") blanks may also be used, *i. e.* samples in which the ascorbic acid is added 5 minutes after the sodium hydroxide. The two types of tissue blanks usually give practically the same values. Standards of adrenaline and noradrenaline as well as a reagent blank are run in parallel with the samples. To control the possible presence of interfering material, known amounts of adrenaline and noradrenaline are added each to one portion of the eluate and treated in the same way as the sample ("internal standards").

Example. The data of a typical determination of the adrenaline and noradrenaline content of a normal rabbit's heart are given below.

The eluate was neutralized to pH 6 and diluted to 14 ml. To a series of test tubes solutions were added as shown in Table III.

The adrenaline ($= x\ \mu g$) and noradrenaline ($= y\ \mu g$) in the sample are calculated by solving the equations:

$(80 - 0.6)\ x + (65 - 0.6)\ y = 74 - 2.0$. (Readings at 410 mμ activ. w.-l.)

$(80 - 1.0)\ x + (14.0 - 1.0)\ y = 20.5 - 2.8$. (Readings at 455 mμ activ. w.-l.)

$$x = 0.05$$
$$y = 1.06$$

Table V.

Differential estimation of adrenaline (A) and noradrenaline (NA) in heart and brain of rabbits.

	No addition		Addition of A or NA to eluate			
	Found μg		Added μg		Found μg	
	A	NA	A	NA	A	NA
Heart I	0.03	1.05	0.10	—	0.13	1.05
Heart II	0.09	1.48	—	0.10	0.08	1.58
Brain I	—0.01	0.25	0.10	—	0.09	0.25
Brain II	—0.01	0.25	—	0.10	—0.01	0.36

Table VI.

Recoveries of noradrenaline (NA) from rabbits' hearts.

Sample no.	No addition (duplicates) μg		Recovery of 5.00 μg NA added to homogenate		extract	
			μg	per cent	μg	per cent
1	4.7	4.9	5.3	106	4.7	94
2	4.3	4.1	4.8	96	5.3	106
3	5.2	5.1	4.7	94	4.8	96

The adrenaline internal standard was found to contain 0.57 μg adrenaline. Subtraction of the sample adrenaline from this value gives 0.52 μg, *i. e.*, 104 per cent of the added adrenaline. The corresponding noradrenaline values were 1.57 μg and 0.51 μg, respectively, *i. e.*, 102 per cent of the added noradrenaline.

Comments: Differential estimations of adrenaline and noradrenaline in various known mixtures (Table IV) and in eluates to which known amounts of adrenaline and noradrenaline had been added (Table V) were carried out according to the method described above. In both types of experiments a reasonably good agreement between expected and observed values was obtained.

Instead of 0.1 M phosphate buffer pH 6.5, 1.0 M sodium acetate buffer, pH 6.0, as used by EULER and FLODING (1955), may be employed. We have chosen always to add $ZnSO_4$ as described above, since it appears to improve the agreement between internal and pure standards.

The fluorophore of DOPA obtained by oxidation and rearrangement in alkali has nearly the same fluorescence intensity as

Table VII.

Recoveries of adrenaline (A) and noradrenaline (NA) from rabbits' brains.

Sample no.	No addition (duplicates)		Recovery of 2.5 μg NA (samples 1—2) or 2.5 μg A (sample 3) added to homogenate		extract	
	μg		μg	per cent	μg	per cent
1	1.44	1.44	2.51	100	2.59	104
2	0.93	0.97	2.62	105	2.47	99
3	0.85[1]	0.85[1]	2.36	94	2.52	101

[1] For technical reasons calculated as adrenaline.

Table VIII.

Recoveries of adrenaline (A) from rabbits' adrenals.

Sample no.	No addition (duplicates)		Recovery of 50 μg A added to homogenate		extract		I/II
	μg		μg	per cent	μg	per cent	
I. Direct fluorescence of catechol amines[1]							
1	94	94	52	104	52	104	1.01
2	100	98	49	98	49	98	0.98
3	120	120	51	102	51	102	1.02
II. Fluorescence of "lutines"[2]							
1	93	94	49.5	99	48.5	97	
2	101	102	49.5	99	48.5	97	
3	118	118	50	100	50	100	

[1] Almost entirely adrenaline.
[2] Almost entirely adrenolutine.

noradrenolutine but has maxima at lower wave-lengths (activation peak 365, fluorescence peak 495 mμ).

Under the present conditions the fluorescence of the fluorophore of dopamine is only about 1.5 per cent of that of noradrenolutine. Under the conditions described by CARLSSON and WALDECK (1958) a stronger fluorescence is obtained. With small variations in techniques the method can thus be used for determination of noradrenaline and adrenaline as well as the precursors of these amines, DOPA and dopamine.

The total catechol amine content of adrenals may be assayed by measuring the fluorescence of the unchanged amines in the ultraviolet region after dilution of the extract 125 to 250 times

Table IX.

Recoveries of adrenaline (A) and noradrenaline (NA) from rabbits' hearts using the simplified extraction procedure.

Sample no.	No addition (duplicates)		Recovery of 5 μg NA (samples 1—2) or 5 μg A (sample 3) added to homogenate 1		homogenate 2	
	μg		μg	per cent	μg	per cent
1	3.7	3.6	4.4	88	4.4	88
2	4.0	4.1	4.9	98	5.1	102
3	4.1[1]	4.1[1]	4.5	90	4.4	88

[1] For technical reasons calculated as adrenaline.

Table X.

Recoveries of adrenaline (A) and noradrenaline (NA) from rabbits' brains using the simplified extraction procedure.

Sample no.	No addition (duplicates)		Recovery of 2.5 μg NA (samples 1—3) or 2.5 μg A (sample 4) added to homogenate 1		homogenate 2	
	μg		μg	per cent	μg	per cent
1	1.05	1.04	2.68	107	2.66	106
2	1.15	1.24	2.43	97	2.50	100
3	1.32	1.31	2.59	104	2.46	98
4	1.20[1]	1.17[1]	2.42	97	2.45	98

[1] For technical reasons calculated as adrenaline.

(DUGGAN *et al.* 1957). That no inhibition of the fluorescence of catechol amines occurs at the dilution used is controlled by internal standards.

Recovery Experiments. Duplicate Determinations.

The homogenates of two brains and two hearts, respectively, were divided into four equal parts. Re-extractions were carried out as described under "Preparation of Extracts". A known amount of noradrenaline (or adrenaline) was added to the homogenate of one of the four portions and to the final extract of another portion. Analysis as described above was then performed. The results of these experiments are shown in Table VI (hearts) and VII (brains). The recoveries of adrenaline added to homogenates and extracts of adrenals are given in Table VIII. In this table a close agreement

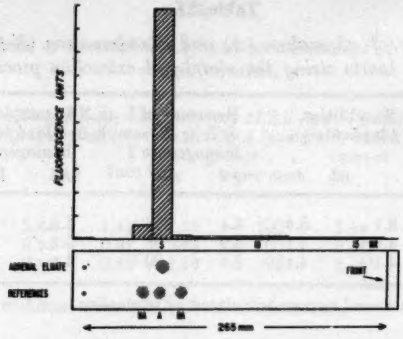

Fig. 7. Quantitative paper chromatography of an adrenal eluate. Activation wave-length 410 mμ. Fluorescent wave-length 540 mμ. For explanation see text.

Note. The activation and fluorescence spectra obtained from piece nr. 5 were almost identical with those of adrenolutine.
The amount of adrenaline recovered from the paper was 4.9 μg.

between measurements of the fluorescence of the unchanged catechol amines and of the lutines is also demonstrated.

As will appear from Tables VI—VIII duplicates generally agree within a few per cent. Recoveries come fairly close to 100 per cent.

The accuracy of the simplified extraction procedure was determined in a similar way. The homogenates of the brains and hearts from two animals were divided into four parts, to two of which known amounts of noradrenaline (or adrenaline) were added. After centrifugation part of the extract, generally 12 ml, was taken for assay (Tables IX and X). Reasonably good recoveries were obtained.

Specificity of the Method.

The following criteria argue for the identity of apparent catechol amines in the eluates with authentic adrenaline and noradrenaline.

1. The fluorescence and activation spectra of the eluates after oxidation and rearrangement in alkali are nearly identical with those of noradrenaline (heart, Fig. 3, and brain, Fig. 4) or adrenaline (adrenals, Fig. 5). The activation and fluorescence spectra of

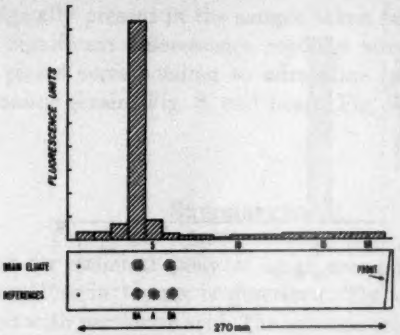

Fig. 8. Quantitative paper chromatography of a brain eluate. Activation wave-length 410 mμ. Fluorescent wave-length 540 mμ. For explanation see text.

Note. The activation and fluorescence spectra obtained from piece nr. 4 were almost identical with those of noradrenolutine. The amount of noradrenaline recovered from the paper was 0.9 μg.

The slight fluorescence, which is almost equally distributed in the majority of the samples is unspecific; it is due to the presence of an impurity in the paper.

nonoxidized adrenal extracts are practically the same as those of catechol amines (CARLSSON *et al.* 1957).

2. For further identification of the substances determined, paper chromatography has been performed. In these experiments the extracts of the adrenals were purified in the same way as described for heart and brain. To the extracts 50 mg of the disodium salt of ethylene diamine tetraacetic acid was always added to minimize the interference of heavy-metal ions. The eluates were evaporated to dryness *in vacuo* and the residues immediately extracted four times with 1 ml acetone containing 1 ml 0.1 N hydrochloric acid per 100 ml. After evaporation almost to dryness, the acetone extract was applied as a spot on a Whatman no. 1 filter paper. The paper was run for 16 to 20 hours at 26° C in n-butanol saturated with N hydrochloric acid, or at about 20° C in phenol-HCl (15 per cent 0.1 N HCl, w/v). It was then dried for about 10 min at 65° C. (When the phenol system was used, the paper was washed with benzene before drying.) The paper was sprayed with a ferricyanide solution (0.44 per cent potassium ferricyanide in 0.1 M phosphate buffer, pH 7.5). Some chromato-

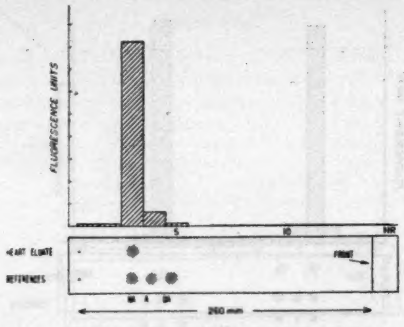

Fig. 9. Quantitative paper chromatography of a heart eluate. Activation wave-length 410 mμ. Fluorescent wave-length 540 mμ. For explanation see text.

Note. The activation and fluorescence spectra obtained from piece nr. 3 were almost identical with those of noradrenolutine. The amount of noradrenaline recovered from the paper was 2.8 μg.

grams obtained in this way are shown in Fig. 6. The eluates of a rabbit's heart give only one spot corresponding to noradrenaline, whereas the brain eluates give two spots, one corresponding to noradrenaline and the other to dopamine. The chromatograms of the adrenals only revealed adrenaline. No special attempts to detect dopamine in these organs have been performed.

Quantitative paper chromatography of the eluates of brain, heart and adrenals has also been undertaken. The eluates of two organs were combined and an aliquot was taken for assay as described above. The remainder was divided into two equal parts, which were run in parallel on a paper as described above using the butanol-HCl system. A mixture of adrenaline, noradrenaline and dopamine (2.5 μg of each) served as a reference. After 16 hours the paper was cut into two parts so that one part of the extract was separated from the other part together with the references. The latter part of the paper was sprayed with potassium ferricyanide as described above. The former part was dried in room temperature for 15 min. It was then cut into small pieces, each of which was eluted with 4 ml 0.01 N HCl. The eluates were assayed for catechol amines fluorimetrically, according to the modified THI method described above. The total amount of catechol amines recovered from the paper was 75 per cent of the

amount originally present in the sample taken for paper chromatography. Significant fluorescence readings were obtained from eluates of pieces corresponding to adrenaline (adrenals, Fig. 7) or noradrenaline (brain, Fig. 8, and heart, Fig. 9).

Summary.

A method for chemical assay of small amounts of adrenaline and noradrenaline in tissues is described. The catechol amines are extracted with perchloric acid. The extracts are passed through a cation exchange column (Dowex 50) which takes up the catechol amines. Elution of the amines from the column is performed by hydrochloric acid. Estimation of the two amines in the eluates is made fluorimetrically after oxidation and rearrangement in alkali. Differentiation between adrenaline and noradrenaline is performed by utilizing the difference in the activation spectra of the fluorophores.

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A Fluorimetric Method for the Determination of Dopamine (3-Hydroxytyramine.)

By

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Dopamine is a probable intermediate in the biosynthesis of noradrenaline and adrenaline (BLASCHKO 1939, 1957). It has been shown to occur in urine (HOLTZ, CREDNER and KOEPP 1942, EULER and HELLNER 1951, EULER, HAMBERG and HELLNER 1951) and in many tissues (see EULER and LISHAJKO 1957, DENGLER 1957, MONTAGU 1957, CARLSSON *et al.* 1958).

Only fluorimetric methods appear to be sensitive enough for the chemical determination of dopamine in tissues and body fluids. The ethylene diamine condensation method of WEIL-MALHERBE and BONE (1952, 1957) can be employed for this purpose, but the fact that the fluorophores of dopamine and adrenaline have almost the same fluorescence characteristics (KÄGI, BURGER and GIGER 1957) limits its usefulness. The method described below is similar to the THI (= trihydroxyindole) method for estimating adrenaline and noradrenaline (EHRLÉN 1948, LUND 1949, EULER and FLODING 1955). In this method the catechol amines are first oxidized to red indole derivatives, adrenochrome and noradrenochrome, respectively, which are then rearranged in alkali to strongly fluorescent trihydroxyindoles, adrenolutine and noradrenolutine, respectively. Previous attempts to estimate dopamine according to this principle have not been successful. The fluorescence obtained has been weak, with activation and fluorescence peaks indistinguishable from

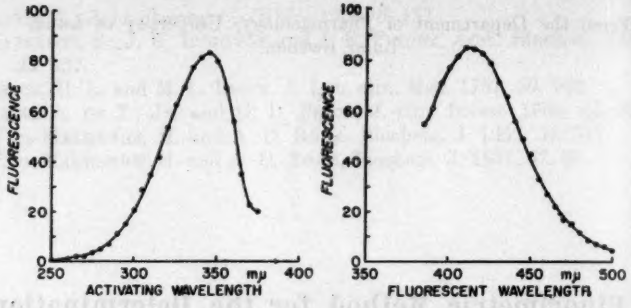


Fig. 1. Activation and fluorescence spectra of the fluorophore of dopamine. Fluorescence is given in arbitrary units. When the activating wave-length was varied, the fluorescent wave-length was set to 410 mμ. When the fluorescent wave-length was varied, the activating wave-length was set to 345 mμ. Concentration of dopamine hydrochloride 0.5 μg/ml.

those of noradrenolutine. With certain modifications of the technique, however, it has been possible to raise the fluorescence obtained from dopamine considerably and also to obtain spectra which differ markedly from those of adrenolutine and noradrenolutine.

Reagents.

0.1 M phosphate buffer, pH 6.5. Dissolve 5.34 g Na_2HPO_4, $2H_2O$ and 9.52 g KH_2PO_4 (Sörensen) in water to a final volume of 1,000 ml.

0.02 N iodine solution. Dissolve 0.254 g iodine and 5 g KJ in 5 ml water and dilute to 100 ml.

5 N sodium hydroxide solution.

Alkaline sulfite solution. Dissolve 5.04 g Na_2SO_3, $7H_2O$ in 10 ml water and dilute with 5 N sodium hydroxide to 100 ml.

5 N acetic acid solution. Dilute 28.5 ml glacial acetic acid with water to 100 ml.

Glass-distilled water is used throughout.

Procedure.

Adjust the pH of the sample to about 6.5. To a silica test tube add 1 to 3 ml sample (0.2 to 2 μg dopamine), 0.5 ml buffer, water to give a total volume of 3.8 ml, and 0.05 ml iodine solution. After 5 min add 0.5 ml alkaline sulfite solution. After another 5 min add 0.6 ml 5 N acetic acid (pH drops to about 5.3). Irradiate

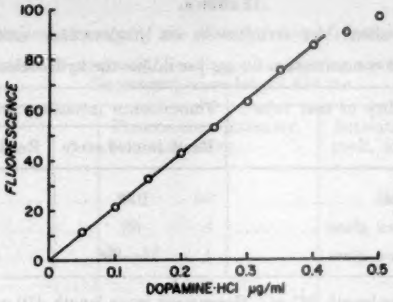


Fig. 2. Fluorescence intensity at varying concentrations of dopamine. Fluorescence is given in arbitrary units. Activating wave-length 345 mμ, fluorescent wave-length 410 mμ.

sample by means of a mercury lamp for 10 min. (The lamp used in the present experiments was "Hanau NN 15/44 VK"; peak emission 254 mμ). Read fluorescence in a spectrophotofluorometer (Aminco-Bowman). Activation and fluorescence peaks (Fig. 1): 345 and 410 mμ, respectively (uncorrected instrumental values). The fluorescence is stable for 24 hours.

A standard and a reagent blank are run together with the sample. When tissue extracts are analysed, a "tissue blank" and an "internal standard" are also run together with the sample. The "tissue blank" is a sample treated as above, except that 5 N sodium hydroxide instead of the alkaline sulfite solution has been added. The "internal standard" is a sample treated as above, except that a known amount of dopamine has been added.

Before tissue extracts are assayed, they must be purified in order to remove interfering substances. A Dowex 50 column is useful for this purpose (see BERTLER, CARLSSON and ROSENGREN 1958).

Comments.

A linear relation was found between the concentration of dopamine up to 0.4 μg per ml and the fluorescence intensity (Fig. 2).

DULIERE and RAPER (1930) observed that dopamine (I) is easily oxidized to a red indole derivative (II), which in the absence of

Table I.

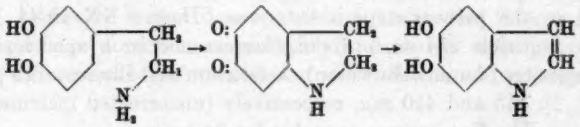
Effect of ultraviolet irradiation on fluorescence intensity.

Dopamine concentration 0.5 μg per ml (as the hydrochloride).

Irradiation min.	Quality of test tube	Fluorescence intensity, arbitraty units[1]	
		Read immediately	Read after 17 hours
10	Silica	100[2]	100[2]
10	Pyrex glass	68	97
—	Pyrex glass	15—20[3]	86

[1] Activating wave-length 345 mμ, fluorescent wave-length 410 mμ.
[2] Set to 100.
[3] Needle unsteady and rising during reading.

oxygen undergoes an intramolecular rearrangement with formation of 5,6-dihydroxyindole (III):



I II III

The latter step was found to be markedly accelerated by the addition of NaOH. The present procedure involves 3 steps, a) oxidation by iodine at pH 6.5, b) rearrangement by addition of the alkaline sulfite solution (the sulfite serves to prevent further oxidation; ascorbate may also be used for this purpose but has the drawback of interfering with the fluorescence), and c) adjustment of pH to about 5.3 by means of acetic acid. The last step serves two purposes, 1) the activating and fluorescent wave-lengths of the fluorophore of dopamine drop much more than those of adrenolutine and noradrenolutine, thus enabling differential estimation of dopamine, and 2) the yield of fluorescent compound appears to increase, as indicated by a slow rise in fluorescence intensity. This rise is hastened by ultraviolet irradiation (Table I).

Among a number of oxidants tested, iodine proved to be the most suitable. Dopamine is not so readily oxidized as adrenaline and noradrenaline. For example, a much higher concentration of potassium ferricyanide is required to oxidize dopamine than is

Table II.

Fluorescence intensity of the fluorophores of catechol derivatives.

Concentration 0.2 μg per ml. Readings at activating wave-length 345 mμ, fluorescent wave-length 410 mμ.

Compound	Fluorescence intensity, arbitrary units	Activation peak, mμ	Fluorescence peak, mμ
Dopamine	50[1]	345	410
Adrenaline	4	—	—
Noradrenaline	4	—	—
Epinine	2	—	—
Dops	56	345	410
Reagent blank	3	—	—

[1] Set to 50.

needed for the other two catechol amines. Experiments with varying concentrations of iodine and varying times of oxidation have been performed. These variables seemed to have no critical influence on the final yield of fluorescent compound.

Specificity. Adrenaline, noradrenaline and epinine (N-methyl dopamine) added in amounts equal to that of dopamine, give readings which hardly differ from that of the reagent blank (Table II). This is due partly to the differences in fluorescence characteristics mentioned above, partly to low yields of adrenolutine, noradrenolutine and the fluorophore of epinine, under the experimental conditions employed.

Dopa (3,4-dihydroxyphenylalanine), when treated as described above, yields a compound with fluorescence characteristics indistinguishable from those of the fluorophore of dopamine. On the other hand, when dopa and dopamine are treated *e. g.* according to the modification of the THI method described by BERTLER, CARLSSON and ROSENGREN (1958), they yield compounds with different fluorescence characteristics. This phenomenon may possibly be explained by the observation of RAPER (1932), that oxidation and rearrangement of dopa may result in two different compounds, one being compound III (see above), the other being 5,6-dihydroxyindole-2-carboxylic acid. In any case, when the purification procedure described by BERTLER *et al.* is used, dopa will not interfere with estimation of dopamine, since it is not taken up by the sodium form of Dowex 50.

Using the procedure described above, tyramine and a number of other substituted phenylalkylamines have been tested. No fluorescence was detectable, however.

Summary.

A fluorimetric method for the determination of dopamine is described. The principle is similar to that employed in the trihydroxyindole method for estimating adrenaline and noradrenaline. Utilizing differences in fluorescence characteristics at pH about 5.3, microquantities of dopamine can be determined in the presence of at least equal amounts of adrenaline or noradrenaline.

This investigation was aided by grants from The Swedish Medical Research Council and from The Swedish Ciba, Ltd. For generous supplies of drugs we are also indebted to F. Hoffmann-La Roche & Co., Ltd., Basle, Switzerland (dopamine and dopa) and Rhône-Poulenc Ltd., Paris, France (adrenaline and noradrenaline).

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Analysis of the Pressure-Time Curves Obtained in the Occluded Femoral Artery in Cats at Elevated Venous Pressure.

By

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In recent years the question has been raised whether distension of the veins in a vascular area influences actively the resistance to flow in the same vascular bed. GASKELL and BURTON (1953), using the plethysmographic technique, found that, when the human limb was lowered below heart level, blood flow in the digits decreased. They concluded that this could only be explained in terms of a reflex effect. The decrease in flow could be elicited from the veins in the digit itself and since the same results were obtained in sympathectomized patients, they concluded that this so-called veni-vasomotor reflex was local in nature. A decreased flow in the 45 degree dependent human limb was also reported by BEACONSFIELD and GINSBURG (1955). YAMADA and BURTON (1954) reported a reduction in blood flow in the finger, when external suction was applied on it. They interpreted this as further evidence for a veni-vasomotor reflex. A reduction in flow in the hand in response to subatmospheric pressure has also been reported by GREENFIELD and PATTERSON (1954) who, however, offered a different explanation for their findings.

Additional support for the concept of a veni-vasomotor reflex has been presented by HADDY and GILBERT (1956) and BURTON and ROSENBERG (1956). On the other hand, contradictory results

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in similar types of experiments have been reported by PHILLIPS, BRIND and LEVY (1955) who found that in the hind limb of the dog flow increased, when the venous pressure was elevated and the arterio-venous pressure difference was maintained by simultaneous elevation of the arterial pressure. Using a heat elimination technique, RODDIE (1955) studied the effect of position on circulation through the fingers. In no experiment was there any evidence for a reduction in finger blood flow of the dependent arm.

Indications of an active mechanism causing an elevation of vasomotor tone, when the venous pressure was locally elevated, had first been obtained by GIRLING (1952) in his study in the rabbit ear of critical closing pressure (CCP, BURTON 1951) and its relation to venous pressure. He found that, when the venous pressure was elevated, the CCP in several instances attained a value higher than the sum of the original CCP and the applied venous pressure and concluded that this effect was due to vasoconstriction elicited by the raised venous pressure. LEVY, PHILLIPS and BRIND (1954), on the other hand, found that in the hind limb of the dog the arterial pressure, at the end of an occlusion period of 10 minutes, attained a value that was practically identical with the applied venous pressure (5—20 cm of water). On the basis of observed changes in CCP in the rabbit hind leg, ROSENBERG (1956) again claimed that elevating the venous pressure produces vasoconstriction by way of a local reflex.

Whether elevation of venous pressure brings about active changes in vasomotor tone in the corresponding area still seems to be an open question. In the present investigation analysis has been made of the pressure-time curves obtained from the distal segment of the occluded femoral artery in cats at different levels of venous pressure.

Methods.

Cats were anesthetized with chloralose (50—60 mg per kg) given intravenously after ether induction. The leg was prepared at mid-thigh level and, after insertion there of polyethylene cannulas in the femoral artery and vein, the muscles were divided by blunt dissection in four groups. After plugging of the bone marrow space of the femur with absorbent paper and ligation of two or three small vessels along the sciatic nerve, four ligatures around the individual muscle groups, and one common ligature, were tightly tied. The vessels were then connected to the measuring unit (Fig. 1) which for the arterial side was principally of the same kind as that used by NICHOL *et al.* (1951).

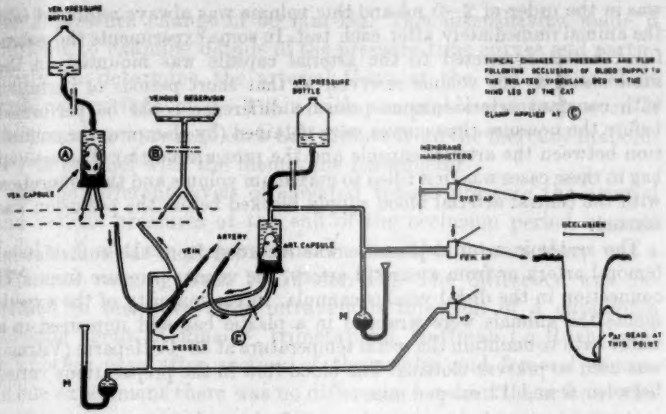

Fig. 1. Shematic representation of recording apparatus. Water transmission in rigid tubes. A and B illustrate the two different methods used to elevate venous pressure. To the right typical results on occlusion of the arterial blood supply (at C) at normal venous pressure are shown. Tracings from above: systemic arterial pressure (syst. AP), femoral arterial pressure (fem. AP) and femoral venous pressure (VP).

Pressure-time curves were obtained in the following manner. The central arterial blood supply to the rubber bag in the water-filled capsule, connected to the mercury and membrane manometers, was occluded for 2—3 min. During the occlusion the blood in the rubber bag was perfused at the pressure in the manometer system which would fall at first sharply and then level out asymptotically towards a horizontal level. The final arterial pressure value after 2—3 min occlusion is termed P_{A_f}. The venous pressure at the same instant is called P_{V_f}. The arterial pressure during the occlusion was recorded photographically by the optic membrane manometer and also read each half minute from the mercury manometer.

The arterial blood flow could be calculated from the slope of the pressure-time curves, since the volume displacement of the manometric system was known. In some cases the venous outflow was measured with a photoelectric drop counter.

Venous pressure was elevated in two different ways. In the early part of the study a capsule with a rubber bag was mounted on the venous side in the same manner as on the arterial side (Fig. 1 A). The capsule was connected to a water-filled bottle and by setting the latter at different heights the resistance for the venous outflow could be varied. In the latter part of the study the venous outflow during the arterial occlusion was collected in a reservoir of wide diameter set at different levels, while the connection to the central side of the vein was clamped (Fig. 1 B). The blood volume collected in the reservoir during each test

was in the order of 2—5 ml and this volume was always reinfused into the animal immediately after each test. In some experiments the water-filled bottle connected to the arterial capsule was mounted on the same stand as the venous reservoir so that short periods of perfusion with constant arterio-venous pressure difference could be performed before the pressure-time curves were obtained (by clamping the connection between the arterial capsule and the pressure bottle). The arterial bag in these cases was first filled to maximum volume and the connection with the central arterial blood supply blocked before the perfusion was started.

The systemic arterial pressure was recorded from the contralateral femoral artery or from a carotid artery, the venous pressure from a T-connection in the distal venous cannula. In the majority of the experiments the animals were wrapped in a plastic bag and immersed in a water bath to maintain the rectal temperature at 38° C. Heparin (Vitrum) was used to prevent clotting. The blood flow in the preparations varied between 8 and 12 ml per min.

Results.

The usual procedure during an experiment was to obtain one or two pressure-time curves at normal venous pressure as determined by the cat's own circulation. If from the photographic record the pressure-time curves were acceptable, the study was then continued at increased levels of venous pressure. To be acceptable the pressure-time curve, after an initial drop, should show a gradual fall in pressure and asymptotically approach a horizontal level. The highest final arterial pressure (P_{Af}) observed at normal venous pressure was 20 mm Hg as reported in a previous communication (YAMADA and ÅSTRÖM, 1959).

In the first series of 12 experiments the venous pressure was elevated by application of hydrostatic pressure from a pressure bottle on the rubber bag in the venous capsule (Fig. 1 A). As implied by GIRLING (1952) and stated by ROSENBERG (1956), the venous pressure was assumed to be determined by the height of the pressure bottle. When later the venous pressure was recorded directly in the vein, this assumption proved invalid. During an occlusion period the venous pressure would usually fall below the value set by the pressure bottle and this fall in venous pressure would cause a progressive fall in arterial pressure. The arterial flow did not become zero within the occlusion times used. The membrane manometer recording the femoral arterial pressure gave a deflection of only 20—28 mm on the photographic paper

for a pressure change of 50 mm Hg. This insensitivity made it difficult to examine details of the pressure-time curves and particularly to determine the arterial flows at the end of the short occlusion periods of 2—3 min with acceptable accuracy, even if the pressures were read each half minute from the mercury manometer in parallel with the membrane manometer.

At the different levels of elevated venous pressure the arterial and venous pressures at the end of the occlusion period became equal in 6 of these preparations. In the other 6 cases there was a difference ranging from 2—20 mm Hg. The difference was decreased in one case after intravenous injection of a ganglionic blocking agent (Arfonad®, trimetaphan, Hoffman-La Roche) and in another case after section of the sciatic and femoral nerves. In one experiment there was no difference between the arterial and venous pressures at the end of the occlusion period at the beginning of the experiment, while such a difference after the same occlusion time was later observed in the same experiment when the resting blood flow was reduced. Owing to the inadequacies of the technique used, the observations in this first series have been considered only preliminary.

In the *second series* of 9 experiments the venous pressure was raised by the same method. The sensitivity of the membrane manometer for the recording of the pressure-time curves was increased so that a pressure change of 8 mm Hg (corresponding to a volume displacement of 0.10 ml) gave a deflection of 15 mm on the photographic paper. "Practically zero" arterial flow was now considered to be present if the flow was less than 0.0067 ml per min. Only pressure values obtained at "practically zero" arterial flow were then used for comparison and only such final arterial pressures are referred to as P_{Af}'s.

In 7 out of the 9 experiments in this series no significant difference between P_{Af} and P_{Vf} was observed. In two cases, however, the P_{Af} at one or more levels of elevated venous pressure was 2 or more mm Hg higher than the P_{Vf}. In both of these experiments Arfonad® abolished the P_{Af}—P_{Vf} difference. The results from all experiments in this second series in which "practically zero" arterial flows were obtained are plotted in Fig. 2 (filled circles). All P_{Af} values obtained in the same experiments at normal venous pressure are also given in the diagram.

In *the third series* of experiments the venous pressure was elevated by a reservoir of a large diameter which was filled with

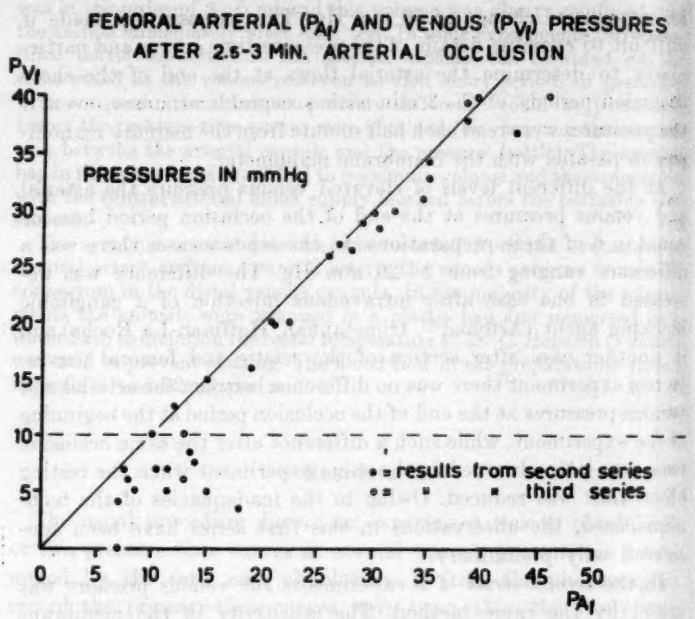

Fig. 2. Plot showing the relationships between P_{Af}'s and P_{Vf}'s obtained after 2—3 min of occlusion. Each dot refers to one animal only.

heparin-Ringer solution and could be set at different heights. The venous outflow from the leg was connected to the reservoir immediately before occlusion of the artery. The amount of blood collecting in the reservoir during a test was not more than 2—5 ml. At the end of the occlusion periods there was a significant difference (more than 2 mm Hg) between P_{Af} and P_{Vf} at the elevated levels of venous pressure in 2 of the 4 experiments. The results in this third series of experiments are also plotted in Fig. 2 (open circles).

During the tests at increased venous pressure in the third series it was regularly observed that, 1—1.5 minutes after the occlusion, there was a retrograde flow from the venous reservoir into the leg. During the whole time the arterial supply was occluded, this retrograde flow amounted to about 2—3 ml at the highest venous pressures (30—40 mm Hg) and it started, while there still was

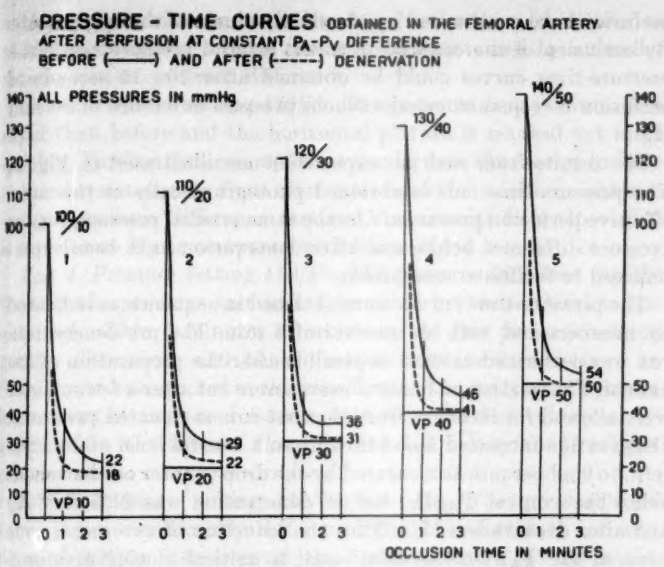

Fig. 3. Pressure-time curves copied from the photographic record and superimposed to facilitate comparison, before (solid curves) and after (dotted curves) denervation. The rate of fall in pressure is more rapid after denervation indicating decreased resistance to flow. The sequence of determinations is indicated by numbers. For further explanation see text.

positive flow on the arterial side, as indicated by the pressure fall in the pressure-time curve at this point. At higher venous pressures some of this retrograde flow was probably due to leakage from small damaged vessels. Part of the retrograde flow, however, would seem to be due to filtration out into the extravascular spaces during these conditions of elevated hydrostatic capillary pressure. This retrograde flow would seem to explain why the elevated venous pressure could not be maintained by the venous capsule arrangement (Fig. 1 A).

In a *fourth series of experiments* the possible role of vasomotor tone for the P_{Af}—P_{Vf} separation at high venous pressure was further studied in experiments in which the venous reservoir was mounted on the same stand as the pressure bottle connected to the arterial capsule. By this arrangement the leg could be perfused by static pressure with blood contained in the rubber bag. The

perfusion time was limited by the small volume of the bag (15 ml). By occlusion of the connection to the arterial pressure bottle the pressure-time curves could be obtained after 10—15 seconds of perfusion at constant arterio-venous pressure difference of 90 mm Hg.

The results from such an experiment are illustrated in Fig. 3. The pressure-time curves obtained photographically at the same effective perfusion pressures, *i. e.* the same arterial pressure/venous pressure difference before and after denervation have been superimposed to facilitate comparison.

The pressure-time curves were obtained in sequence as indicated by numbers and with an interval of 3 min. The pre-denervation run was performed as soon as possible after the preparation of the animal. The sciatic and femoral nerves were cut after a few minutes were allowed for recovery from the first run at elevated pressures. Denervation increased blood flow, from a control level of 5 ml per min, to 9 ml per min as measured by the drop counter on the venous side. The control P_{Af}/Pv_f before denervation was 21/5 mm Hg and after denervation 17/4. Thus the reduction of vasomotor tone lowered the P_{Af} (in this case equal to critical closing pressure). For a reduction of 3 mm Hg in the minimum arterio-venous pressure difference required for flow, the resting blood flow at the prevailing systemic arterial pressure increased by 80 per cent.

To facilitate description of the pressure-time curves in Fig. 3 the assumption that vessels, including the resistance vessels, are distensible will be made. The support for such an assumption will be dealt with in the Discussion.

The pre-denervation run (solid curves) was performed at relatively high vasomotor tone. This high tone was indicated by the observation that denervation in this particular preparation increased blood flow considerably more than usually observed.

Test 1. Pressure setting 100/10 mm Hg. Upon occlusion pressure falls relatively slowly and the criterion of "practically zero" flow (less than 0.0067 ml per min) is barely met at the end of 3 min. P_{Af} equals 22 mm Hg and the P_{Af}-Pv_f difference 12 mm Hg.

Test 2. Pressure setting 110/20 mm Hg. There is a more rapid fall in pressure and the terminal horizontal level is reached more quickly. Thus the distension of the vessels at this higher pressure setting seems to have decreased the resistance to flow (which is a function of the rate of fall in pressure). P_{Af} equals 29 mm Hg. The P_{Af}—Pv_f difference is decreased by 3 mm Hg, as compared

with test 1, and is now 9 mm Hg. In other words, with distension of the vascular bed arterial flow can be maintained by a smaller arterio-venous pressure difference.

Test 3. Pressure setting 120/30. The pressure drop is even more rapid than before and the horizontal plateau is reached yet more quickly. Further distension of the vascular tree thus seems to have reduced resistance to flow even more and the minimum arterio-venous pressure difference required for arterial flow (P_{Af}—P_{Vf}) is now 6 mm Hg as compared with 9 mm Hg in test 2.

Test 4. Pressure setting 130/40. The pressure falls less rapidly than in the previous test and a terminal horizontal level is not obtained within 3 minutes of occlusion. At this pressure setting greater distension, and hence further decrease in resistance to flow, would be expected in this case but, on the contrary, the curve here shows an increased resistance to flow. No P_{Af} value can be given, since the arterial flow did not fall below 0.0067 ml per min.

Test 5. Pressure setting 140/50 mm Hg. The pressure-time curve is not significantly different from that of test 4, *i. e.* any signs of further distension at the higher pressure setting is not revealed. Again, no P_{Af} value can be given.

In the last three tests retrograde flow from the reservoir was observed as usual. The retrograde flow increased with venous pressure but did not exceed 2—3 ml at the venous pressure of 50 mm Hg. Exact measurement of the volumes was not made. No leak was detectable. The variance from the results expected from an assumption of distensibility in tests 4 and 5 will be discussed below. It would seem, however, that, even if a limit for distension had been reached already in test 3, a new factor must have entered, since resistance to flow in tests 4 and 5, in spite of the higher intravascular pressure, actually increased above the value in test 3, as is evident in the rate of fall in pressure.

The post-denervation run (dotted curves) at all comparable pressure settings, shows more rapid initial fall in pressure than that seen in the pre-denervation run. In all tests terminal horizontal levels of arterial pressure are reached and these levels are all lower than before. A P_{Af}—P_{Vf} difference of a significant magnitude is seen only in test 1 (8 mm Hg), and possibly in test 2, while in the other tests it may be said that the P_{Af}'s equal the corresponding P_{Vf}'s. In these last tests the equalization of the arterial pressures to the applied venous pressures accounts for the shape

of the terminal parts of the pressure-time curves since the venous pressure was maintained by the reservoir at a constant level all during the occlusion period. Adopting again the assumption that the vessels are distensible, it may be said that while in test 1 an arterio-venous pressure difference of 8 mm Hg is required to sustain flow, in the other tests at higher pressure settings the increased caliber of the vessels (due to distension) seems to allow flow continuously down to "practically zero" pressures differences.

Discussion.

The pressure-time curves recorded in the distal segment of the occluded femoral artery have shown that at elevated venous pressure (above 10 mm Hg) the arterial pressure, at the end of an occlusion period of 2—3 minutes, usually becomes identical with the venous pressure. In 5 out of 14 cases, however, there was a separation between P_{Af} and P_{Vf} at the end of the occlusion period, when arterial flow was "practically zero" (less than 0.0067 ml per min). The maximum P_{Af}—P_{Vf} difference observed was 6 mm Hg. In no case was the final arterial pressure at elevated venous pressure greater than the sum of the original P_{Af} (determined at normal venous pressure below 10 mm Hg) and the applied venous pressure. In cases where the terminal arterial and venous pressures became equal there would, of course, be no indication of a "critical closure" and in such instances the term P_{Af} would not correspond to critical closing pressure. The observation that in most cases at elevated venous pressure the P_{Af}'s and the P_{Vf}'s became equal is in contrast to the relationship existing at low venous pressure. It has previously been shown that in the venous pressure range below 10 mm Hg the vasomotor tone and tissue pressure seem largely to determine the arterio-venous pressure difference at which arterial flow ceases (YAMADA and ÅSTRÖM, 1959).

In discussing the possible mechanisms involved in the type of experiments presented here, it seems relevant first to consider the effect of increased intraluminal pressure on the caliber of the resistance vessels. A detailed discussion of this debated question (cf FOLKOW 1952, 1956) is beyond the scope of the present study but recent investigations (PHILLIPS, BRIND and LEVY 1955, FOLKOW and LÖFVING 1956) have supported the conclusions by GREEN

et al. (1944) that the resistance vessels are distensible and that resistance to flow in a peripheral vascular bed will decrease, if parallel elevation of the arterial and venous pressures is performed. An increase in flow at higher vascular pressures has been observed several times in the present investigation. In view of available data the assumption of distensibility used in the description of the pressure-time curves in Fig. 3 thus seems to have considerable support. According to HOWELL and RICHARDS (1955) this distensibility is dependent on vascular tone.

The effect of elevated venous pressure *per se* in a vascular bed should also be discussed. Increased venous pressure will accelerate net capillary filtration and ultimately lead to an increase in tissue pressure. As discussed *i. a.* by PHILLIPS, BRIND and LEVY (1955), the cross-sectional dimension of a distensible tube is dependent upon the difference in pressure across its wall. The diameter of the vessels will thus tend to diminish, if under elevated venous pressure tissue pressure increases and counteracts the force of distension within the resistance vessels. Changes in pressure-time curves observed after elevation for some time and then lowering of the venous pressure will be discussed elsewhere (YAMADA and ÅSTRÖM 1959) and explained as being due to a persisting increase in tissue pressure produced by the preceding elevation in venous pressure. In the present investigation this effect was observed in all four cases in which pressure-time curves were obtained up to 5 min subsequent to elevation of the venous pressure to 40—50 mm Hg for 4—5 min.

In the type of experiments described here the arterial pressure at the end of the occlusion period cannot fall below the value of the raised venous pressure. Assuming that the vessels are distensible this would leave the resistance vessels with a larger caliber at the end of an occlusion period at elevated venous pressure. At low venous pressure, on the other hand, the distending force will be gradually reduced (as pressure falls) to a point at which "critical closure" may occur; or, the caliber of the vessels becomes so small that zero arterial flow will be observed, while there still remains an appreciable arterio-venous pressure difference. The presence of a greater force distending the resistance vessels from the inside at elevated venous pressure would, as stated above, in many cases seem to be counteracted by the simultaneous increase in tissue pressure. The resistance to flow in such cases would be determined by the balance between these forces and the vasomotor tone. If

venous pressure is high, or moderate but increased for a prolonged period of time, the effect of tissue pressure would seem to deserve consideration in flow studies as well as in determination of pressure-time curves as performed in this investigation. It may seem reasonable to assume that the role of increased tissue pressure in such cases will be relatively more important, if high vasomotor tone be present at the same time and the active tension of the walls of the resistance vessels thus balances the distending force. If vasomotor tone be low, on the other hand, an increased intravascular pressure might have distended the resistance vessels nearly maximally and in such cases a few mm Hg rise in tissue pressure would probably not affect the caliber of these vessels appreciably.

These considerations seem to explain best the results of the experiment illustrated in Fig. 3 as well as the results of the other 4 cases in which a P_{Af}—P_{Vf} difference persisted at elevated venous pressure. The decrease in terminal arterio-venous pressure difference at the end of the occlusion observed with successive elevation of pressure in Fig. 3, both before and after denervation, would be explained by the distension of the resistance vessels. The lack of further decrease in this difference at the highest pressure settings (tests 4 and 5) before denervation would seem to be explained by a counteracting effect of increased tissue pressure. After denervation the active tension in the walls of the resistance vessels is reduced and the intraluminal pressure is more effective in increasing the caliber of the vessels. As a result the minimum arterio-venous pressure diffference required for flow is reduced to practically zero already in test 2. No evidence of increased resistance to flow is observed at the highest intravascular pressures in this post-denervation run, even if the increase in tissue pressure would be expected to be similar to that in the pre-denervation run. This would seem explicable, if the resistance vessels after denervation become almost maximally distended by the highest intravascular pressures used.

In general a persisting P_{Af}—P_{Vf} difference at elevated venous pressure, as observed in 5 of 14 cases, would thus seem to be expected under conditions of high prevailing vasomotor tone. In such cases procedures reducing vasomotor tone would abolish or diminish the P_{Af}—P_{Vf} difference. This has been shown to be the case in the present investigation following denervation and administration of a ganglionic blocking agent. The occasionally observed P_{Af}—P_{Vf} difference at elevated venous pressure may thus be explained without any assumption of a reflex mechanism. It may

be argued, however, that the increase in resistance to flow sometimes observed at very high intravascular pressures, as illustrated in the last two tests in the pre-denervation run in Fig. 3, is due to a reflexly induced increase in vasomotor tone in accordance with the concept of a veni-vasomotor reflex. Here, an increase in tissue pressure has been considered more likely in view of the following observations: (1) a pressure-time curve obtained at normal venous pressure up to 5 minutes subsequent to a series of tests at elevated venous pressure often shows increased resistance to flow (decreased rate of fall in pressure) and also an elevation of P_{Af}. This was observed regardless of whether the preceding series of tests at elevated venous pressure had shown a separation between the P_{Af}'s and the corresponding P_{Vf}'s; and (2) the increased resistance to flow and the elevation of P_{Af} observed here subsequent to raising the venous pressure during blood perfusion to 40—50 mm Hg can in the same period of time (about 4 minutes) be produced at lower venous pressure, with Ringer perfusion (YAMADA and ÅSTRÖM 1959).

The difference in results obtained in this investigation as compared with those of GIRLING (1952) and ROSENBERG (1956), particularly regarding the magnitude of the P_{Af}—P_{Vf} difference sometimes observed at elevated venous pressure, would seem explicable largely on the basis of differences in technique used. The tissue pressure as a possible factor increasing the resistance to flow under certain conditions of elevated venous pressure would seem to warrant consideration in the study by HADDY and GILBERT (1956), before any definite conclusions can be drawn as to the existence of a "veni-vasomotor reflex" in the dog.

If the interpretation of the role of tissue pressure as a factor counteracting the distension of the resistance vessels at high intravascular pressures be correct, it would not seem unlikely that this factor of tissue pressure might, at least in part, explain some of the differences in results previously obtained regarding the distensibility of the vessels. It has been concluded in this investigation that even small changes in tissue pressure under conditions of high vasomotor tone may have a considerable influence on the caliber of the vessels and, hence, on the resistance to flow.

Summary.

1. Analysis of the pressure-time curves obtained in the distal segment of the occluded femoral artery in cats under chloralose anesthesia has been performed at different venous pressures.

2. Experimental results have supported the concept that the resistance vessels are distensible and that resistance to flow will decrease, if intravascular pressure is increased. In accordance with this the arterio-venous pressure difference required to maintain flow becomes progressively less with increasing intravascular pressure and at venous pressure of 20—30 mm Hg this difference usually approaches zero. When the resistance to flow is high (due to high prevailing vasomotor tone and/or high tissue pressure), arterial flow may, however, sometimes cease, when there still is an appreciable arterio-venous pressure difference towards the end of an occlusion period at elevated venous pressure. This has been observed in 5 of 14 cases. In such cases the arterio-venous pressure difference can be reduced or abolished by measures that reduce vasomotor tone (denervation or administration of a ganglionic blocking agent).

3. The usually observed effect of distension of the resistance vessels at elevated venous pressure may, in cases with high vasomotor tone, tend to be masked after some time by the counteracting effect of increased tissue pressure which results from increased filtration into the tissue. At low vasomotor tone (after denervation) this counteracting effect has not been demonstrable.

4. This investigation has yielded no evidence for the existence of a "veni-vasomotor reflex" in the hind leg of the cat.

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Die Ausscheidung von Methylenblau in den Magensaft.

Von

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Eingegangen am 26. Juni 1958.

Im Jahre 1908 machte FULD die Beobachtung, dass Neutralrot, in den grossen Magen eines Pavlov-Hundes gebracht, auch im kleinen Magen erschien. Seitdem ist in mannigfachen Untersuchungen der Versuch unternommen worden, dieses Phänomen zu erklären.

Einen guten Überblick über die bis 1945 über dieses Thema erschienene Literatur geben KOLM, KOMAROV und SHAY (1945). Es fehlt auch nicht an Versuchen, sich dieses Phämomens, dass Farbstoffe, dem Menschen injiziert, von besonderen Organen, z. B. dem Magen, bevorzugt wieder ausgeschieden werden, für klinische Zwecke zu bedienen. HALLÉN (1948) z. B. beobachtete, dass in einigen Fällen von Hyperazidität die Zeit, in der der Farbstoff (Neutralrot) im Magensaft erschien, kürzer als normal war, während er in Fällen von Achylie überhaupt nicht im Magensaft auftrat.

Verschiedene Autoren wie KOBAYASHI (1935), INGRAHAM und VISSCHER (1935), RAY (1951) sind der Ansicht, dass Faktoren der elektrischen Ladung den Durchgang eines Farbstoffes durch die Zelle bestimmen. Dabei tragen die im Magensaft erscheinenden Farbstoffe ihr Chromogen im elektropositiven Ion, sind also basischer Natur. Sie gehören hauptsächlich zu den Thiazinen, den Oxazinen und den Azinen. Farbstoffe von saurer Natur erscheinen dagegen im Pankreas. MORRISON, REEVES und GARDNER

(1936) geben allerdings an, dass auch saure Farbstoffe im Magensaft erscheinen. Der Gegensatz zwischen ihnen und den oben erwähnten Autoren ist wohl daraus zu erklären, dass beide Autorengruppen verschiedene Methoden für die Stimulierung der Magenzellen benutzen, die ja die Salzsäure produzieren, sowie auch alle anderen Magendrüsen, die aktiv an der Bildung des Magensaftes beteiligt sind. SHORE, BRODIE und HOGBEN (1957) nehmen eine Lipoidbarriere zwischen Blutplasma und Magen an, durch die nur die undissoziierten Moleküle passieren könnten. Die Ionisationskonstante würde also bei dieser Theorie eine Rolle spielen. KOBAYASHI wies aber schon 1935 darauf hin, dass die Farbstoffausscheidung nicht allein durch Diffusionsfähigkeit oder Lipoidlöslichkeit erklärt werden kann. Dass die Chromophore immer eine — N-Gruppe, als Auxochrome immer eine Amino-, niemals aber eine Sulfogruppe besitzen und dass sie als Farbstoffbasen Salze bilden, schien ihm wichtig. Alle diese angeführten Autoren versuchten, das Problem vom rein qualitativen Standpunkt zu lösen. Diejenigen aber, die quantitativ versuchten, die Farbstoffausscheidung zu bestimmen, scheiterten an den von ihnen angewandten Methoden. Gewöhnlicherweise injizierten sie nämlich Histamin in Form von Bienenwachsdepots, die Farbstoffe wurden intramuskulär gespritzt. Diese Art der Stimulation und der Farbstoffinjektion macht die quantitative Bestimmung der Ausscheidung unmöglich oder erschwert sie mindestens, da Histamin- und Farbstoffkonzentration während der Dauer des Versuches nicht konstant bleiben.

ÖBRINK hat nun 1948 die Kinetik von Neutralrot als dem bis dahin am häufigsten verwendeten Farbstoff studiert. Diese Arbeit ist bis heute die einzige, die das Problem der Farbstoffausscheidung rein quantitativ angeht und die mittels der angewendeten Methodik Ergebnisse liefert, die die Diskussion um die Farbstoffausscheidung wieder in Gang setzen wird.

Die vorliegende Arbeit ist eine Fortsetzung der von ÖBRINK begonnenen Studien über die Kinetik der Farbstoffe im Organismus unter besonderer Berücksichtigung des Magens.

Methodik.

Die meisten Versuche wurden an einem 12 kg schweren Heidenhain-Hund ausgeführt, einige andere an einem 22 kg schweren. Die vom zweiten Hund erhaltenen Ergebnisse können aber, rein qualitativ gesehen, mit denen des ersten verglichen werden.

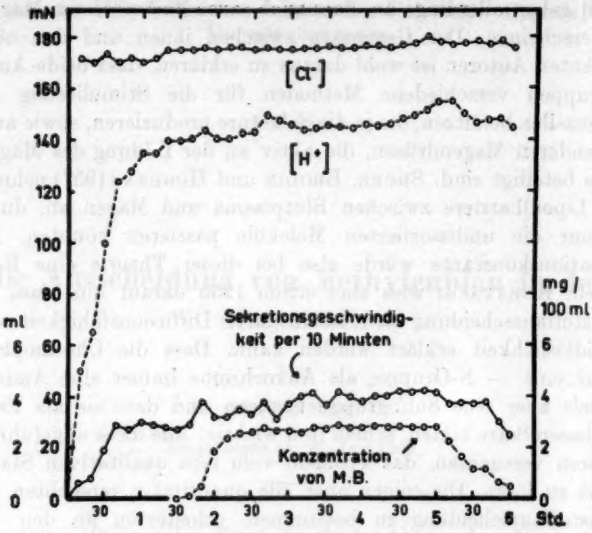

Fig. 1. Ein typisches Experiment mit kontinuierlich intravenöser Injektion von Histamin und Methylenblau. Sobald die Sekretionsgeschwindigkeit konstant war, wurde mit der Injektion von Methylenblau begonnen (1.30 Uhr). Die Injektionen von Methylenblau wurden um 5.00 Uhr beendet, von Histamin um 5.40 Uhr.

Histamin wurde als Histamin-Dihydrochlorid mit Hilfe eines Dauerinjektionsapparates nach ÖBRINK (1948) intravenös injiziert. Methylenblau (MERCK, Nr. 6040, Molekulargewicht: 319.84) wurde in Wasser gelöst, 1 g auf 100 ml, gefiltert und sterilisiert. Die endgültige Stärke dieser Lösung betrug 0.86 %. Um eine konstante Konzentration von Methylenblau (M.B.) im Blute zu gewährleisten, war es nötig, den Farbstoff ebenso wie Histamin mit Hilfe des Dauerinjektionsapparates kontinuierlich intravenös zu injizieren. Dabei wurde folgendermassen vorgegangen: Sobald durch die Histaminstimulation konstante Volumina von Magensaft erhalten wurden, sobald also ein »steady-state» eingetreten war, wurde der Farbstoff injiziert. Der Versuch wurde beendet, oder eine neue Phase des Versuches wurde begonnen, wenn der Magensaft eine konstante Farbkonzentration aufwies.

Der Magensaft wurde durch einen mit einer Art Bürettenhahn versehenen Messzylinder aufgefangen, der durch einen Gummischlauch mit der Silberkanüle des Heidenhainmagens verbunden war. Alle zehn Minuten wurde die Sekretionsrate bestimmt und Proben für die chemische Analyse entnommen.

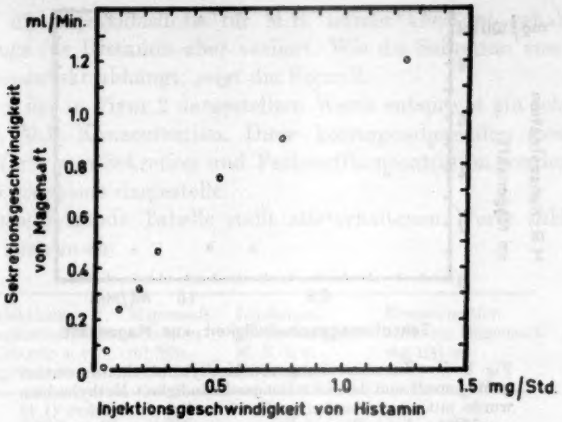

Fig. 2. Der Zusammenhang von Injektionsgeschwindigkeit von Histamin und der Sekretionsgeschwindigkeit von Magensaft.

Die Bestimmung der Säure.

a) Gesamtazidität:

0.1 ml Magensaft wurden 4 ml H_2O und Bromthymolblau als Indikator zugesetzt (pH-Bereich: 6.0—7.6) und gegen 0.01 N NaOH titriert. Die Stärke der Natronlauge wurde jeden Tag mit 0.1 N HCl kontrolliert; als Blindwert wurde die Neutralisation der Natronlauge durch 4 ml H_2O bestimmt.

b) Freie Azidität:

Auf die Bestimmung der freien Säure konnte verzichtet werden, da durch die angewandte Technik mit Histamin der Unterschied zwischen totaler und freier Säure sehr gering war.

Die Chlorid-Bestimmung.

Für die Chlorid-Titration konnten dieselben Proben verwendet werden, die schon zur Bestimmung der Gesamtazidität gedient hatten. Sie wurden jeweils mit 2 ml 0.1 N H_2SO_4 versetzt und dann elektrometrisch mit 0.005 N $AgNO_3$ titriert.

Die Konzentrationsbestimmung von Methylenblau im Magensaft.

Da die Absorptionskurven eines Farbstoffes bei verschiedenem pH verschieden sind, d. h. unterschiedliche Absorptionsmaxima aufweisen, war es notwendig, den »isosbestischen Punkt« für M.B. zu bestimmen. Zu diesem Zweck misst man die Absorptionskurven einer gleichen Konzentration von M.B. bei verschiedenen pH-Werten im Beckmann-Spectrophotometer.

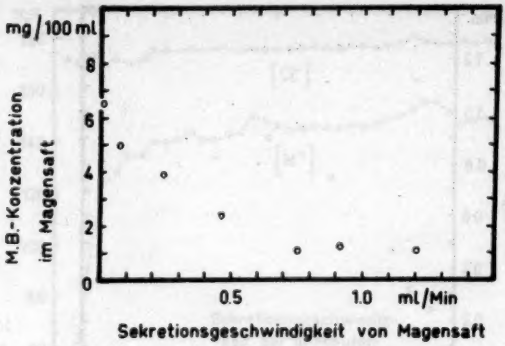

Fig. 3. Der Zusammenhang von Methylenblaukonzentration in Magensaft und der Sekretiongeschwindigkeit. Methylenblau wurde mit einer konstanten Geschwindigkeit injiziert (1.25 ml/Std.). Jeder Wert stellt einen steady-state Wert dar.

Der »isosbestische Punkt« liegt für M.B. bei 672 mμ.

Die Extinktionswerte der einzelnen Proben wurden mit einem Coleman-Junior-Elektrophotometer bestimmt.

Da M.B. in Leuco-Form ausgeschieden wird, wurden die Proben erst nach 24 Stunden analysiert, um ganz sicher zu sein, dass aller Farbstoff oxydiert worden war.

Ergebnisse.

Mit der ÖBRINKschen Technik wurden Histamin und M.B. kontinuierlich intravenös injiziert. Die Figur 1 zeigt, dass ein steady-state für die Magensekretion 30 bis 60 Minuten nach Beginn des Versuches erreicht wird. Dann wird M.B. injiziert, das seinen steady-state nach etwa 50 bis 60 Minuten erreicht. Die Injektion von M. B. geschah ausreichend lange, um eine konstante Farbkonzentration im Magensaft zu gewährleisten. Dann wurde der Versuch beendet oder eine neue Phase des Versuches begonnen.

Die Figur 1 zeigt einen leichten Sekretionsanstieg von ungefähr 0.1 ml/Min., nachdem M.B. injiziert worden ist. Diese Beobachtung machten schon KOLM, KOMAROV und SHAY (1945) und ÖBRINK (1948) beim Neutralrot. Ob dieser stimulierende Effekt auf dem Farbstoff selbst, oder auf in ihm enthaltene Unreinheiten beruht, ist nicht ganz klar.

In den acht auf diese Art und Weise durchgeführten Versuchen

wurde die Injektionsdosis für M.B. immer konstant gehalten, diejeninge für Histamin aber variiert. Wie die Sekretion von der Histaminzufuhr abhängt, zeigt die Figur 2.

Jedem der in Figur 2 dargestellten Werte entspricht ein solcher für die M.B.-Konzentration. Diese korrespondierenden steady-state Werte von Sekretion und Farbstoffkonzentration werden in Figur 3 graphisch dargestellt.

Die nachfolgende Tabelle stellt alle erhaltenen Werte zahlenmässig zusammen:

Injektionsgeschwindigkeit Histamin i. v. mg/Stunde	Magensaftsekretion ml/Min.	Injektionsgeschwindigkeit M. B. i. v. ml/Stunde	Konzentration M.B. im Magensaft mg/100 ml
0.025	0.016	1.25	6.50
0.05	0.078	1.25	5.04
0.1	0.24	1.25	3.97
0.25	0.463	1.25	2.46
0.50	0.754	1.25	1.15
0.75	0.906	1.25	1.37
1.25	1.20	1.25	1.23

Diskussion.

Die Versuche, die rein quantitativ durchgeführt wurden, haben gezeigt, dass Methylenblau, intravenös injiziert, im Magensaft eines Heidenhain-Hundes erscheint. Mit Hilfe der besonderen Technik konnten sowohl die Konzentration von M.B. im Magensaft wie auch das Sekretionsvolumen selbst in jeder gewünschten Weise konstant gehalten werden. So wurde es möglich, die Beziehungen zu untersuchen, die zwischen Sekretion und Farbstoffausscheidung bestehen. Die Figur 3 zeigt, dass die Konzentration von M.B. im Magensaft sich relativ konstant hält bei mittleren und grossen Sekretionsgeschwindigkeiten, dass sie aber bei kleinen Geschwindigkeiten steil ansteigt.

Dieses Ergebnis steht in völliger Übereinstimmung mit dem, was Öbrink (1948) für Neutralrot gefunden hat.

Bei Untersuchungen zur Primäraziditität, die Linde, Teorell und Öbrink (1947) anstellten, ergab sich, dass sie in Abhängigkeit zur Sekretionsgeschwindigkeit und damit zum Sekretionsvolumen steht, d. h., dass sie bei grossen Magensaftvolumina niedrig ist (ungefähr 170 mN), bei geringen und extrem kleinen

aber steil ansteigt. Man ist versucht, diese Kurve für die Primäraziditität mit derjeningen in Figur 3 zu vergleichen. Beide zeigen ungefähr den gleichen Verlauf. Aber ein solcher Vergleich hat Bedeutung nur dann, wenn man weiss, dass die Blutkonzentration in beiden Fällen konstant war. Schon bei den Untersuchungen zur Natur der Ausscheidung von Neutralrot jedoch hatte es sich als ungemein schwierig erwiesen, den Farbstoff im Blutplasma nachzuweisen. Bei der vorliegenden Untersuchung wurde deshalb auf den erneuten Nachweis verzichtet.

Es scheint jedoch mit KOLM *et al.* (1945), ÖBRINK (1948) und RAY (1951) so zu sein, dass infolge der pH-Differenz von Blut und Magensaft eine enorme Akkumulation des Farbstoffes in der Mucosa des Magens Platz greift. Die Interzellularräume sind zwar nicht frei von Farbstoff, er findet sich dort nur in weit geringerer Konzentration.

Dass zwei so chemisch voneinander verschiedene Farbstoffe wie Neutralrot und Methylenblau auf die gleiche Weise ausgeschieden werden, könnte Anlass zur Verwunderung geben. Auf Grund des grossen Akkumulationsgrades von Neutralrot und wahrscheinlich auch von Methylenblau, ist man versucht, an einen besonderen Sekretionsprozess zu denken, der in diesem Falle vorliegen könnte. Ein solcher aktiver Transport würde aber begreiflicherweise auch einen besonderen Transportmechanismus erforderlich machen. Die Affinität zu so verschiedenen Molekülen macht aber eine derartige Annahme zweifelhaft. Nur gleichartige Versuche mit anderen Farbstoffen können hier endgültige Klarheit schaffen.

Dass die in den Magensaft ausgeschiedenen Farbstoffe alle basischer Natur sind, würde für die Existenz einer Lipoidmembran sprechen, die sich zwischen Blutplasma und Magenzelle befinden würde. Durch sie hindurchdringen könnten nur die undissoziierten Farbstoffmoleküle. Auf der sauren Seite würden sie dissoziieren und damit dann für die Membran impermeabel werden.

Die Tatsache, dass die Farbstoffkonzentration im Magensaft umso grösser wird, je kleiner die Sekretionsgeschwindigkeiten sind, kann wohl mit ÖBRINK (1948) so erklärt werden, dass bei niedrigen Sekretionsgeschwindigkeiten eine vollständigere Extraktion des Farbstoffes von dieser Lipoidmembran auf Grund des längeren Kontaktes zwischen ihr und dem Magensaft stattfindet.

Zusammenfassung.

1. An Heidenhain-Hunden wird die Ausscheidung von Methylenblau rein quantitativ untersucht.

2. Es wird nachgewiesen, dass die Konzentration des Farbstoffes im Magensaft von der Sekretionsgeschwindigkeit abhängig ist. Die Akkumulationstheorie und die Existenz einer Lipoidmembran versuchen dieses Ergebnis zu erklären.

Summary.

1. Quantitative studies on the elimination of methylene blue through the gastric mucosa were performed on dogs with Heidenhain pouches.

2. It could be demonstrated that the concentration of the dye in the gastric juice depended on the rate of volume sceretion. Its accumulation in the gastric juice could possibly be explained by the existence of a lipoid membrane through which the dye was eliminated.

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Investigations on the Gizzard Lining of the Chick.

By

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As far as we are aware, a study of the chicken gizzard lining[1] with respect to its amino acids and pigment has not yet been made with modern methods. We have, therefore, carried out the following investigations on this subject.

The protein of the lining.

Experimental.

Collection and preparation of the material for analysis.

The gizzard from several hundred chicks, 4 to 8 weeks old, reared for various experimental purposes on commercial and artificial diets, were taken out when the birds were autopsied. The linings, all of which were yellow and without ulcers, were separated and briefly rinsed with water. They were then dried in a current of air at about 28° C, whereby they became brittle and could be broken into small pieces. The material was extracted with ether in a Soxhlet apparatus. The ether extract which amounted to 0.85 % of the dry material was discarded. Part of the ether-extractable material may have originated from contamination with food constituents. The defatted substance which contained 7.55 % H_2O was powdered and dried in vacuo over P_2O_5 at 60° C to constant weight.

[1] Synonyms: horny layer, stratum corneum, keratinoide Schicht, Koilinschicht.

Table I.

Composition of the gizzard lining.

	% of dry matter	Nitrogen % of dry matter
	(corrected for 7.55 % H_2O)	
Alanine	4.43	0.70
Arginine	5.68	1.83
Aspartic acid	11.19	1.18
Cystine	1.53	0.18
Glutamic acid	11.30	1.08
Glycine	5.01	0.94
Histidine	3.32	0.90
Leucine	10.50	1.12
Lysine	2.65	0.51
Methionine	1.29	0.12
Phenylalanine	3.59	0.30
Proline	4.22	0.51
Serine	3.80	0.51
Threonine	4.67	0.55
Tryptophan	0.45	0.06
Tyrosine	6.65	0.51
Valine	5.71	0.68
	85.99	11.68
Amide-nitrogen	1.22	
Nitrogen in pigment, extracted with acid methanol	0.03	
Nitrogen (total)	13.37	
% nitrogen, accounted for	96.8	
Fat (Schmid-Bondzynski-Ratzlaff)	0.34	

Determination of amino acids, total nitrogen, residual fat, and qualitative test for carbohydrates.

The amino acids, except tryptophan, were determined by paper chromatography of the N-2,4-dinitrophenyl derivatives as described by KOCH and WEIDEL (1956). Tryptophan was determined by the method of SPIES and CHAMBERS (1949).

For the paper chromatography, approximately 5 mg of the powdered material was hydrolyzed with 1 ml of a mixture of equal volumes of 6 N hydrochloric acid and 90 % formic acid for 24 hours at 100° C. The hydrolysis was carried out in an atmosphere of nitrogen in sealed tubes placed in boiling water.

Total nitrogen was determined after digestion of the material with concentrated sulfuric acid and potassium permanganate according to BEET (1955) followed by steam distillation as described by PREGL (1945). The ammonia was absorbed in 4 % boric acid and titrated with N/100 HCl according to MEEKER and WAGNER (1933). Amide nitrogen was determined by the method of FOWDEN (1954).

Determination of residual fat (fat not extracted by the Soxhlet treatment) was carried out by the method of SCHMID, BONDZYNSKI and RATZLAFF as described by FLEISCHMANN-WEIGMANN (1935).

The results of these determinations are presented in Table I.

A qualitative test for carbohydrate by the Molisch test with α-naphthol and concentrated sulfuric acid was negative when carried out on the hydrolyzed material, but positive when made on an extract prepared by boiling the powdered, dried gizzard lining with water. After several extractions with boiling water, the Molisch reaction was negative in the last extract. However, when thereafter the material was boiled with 5 ml water plus 5 drops of conc. sulfuric acid, the acid extract gave a positive Molisch reaction. This seems to indicate that the positive reaction was not entirely due to contamination with carbohydrate from the food.

The solubility properties of the protein together with its amino acid composition indicate that it is a scleroprotein (albuminoid), the cystine content of which is much lower than that of most keratins[1], whereas the glycine content is much lower than the glycine content of collagen and elastine. This scleroprotein represents the somewhat hardened secretion of the glands in the *stratum glandulare*. STREESEMANN (1927—1934) has used the term "Koilinschicht" for the gizzard lining. In accordance herewith, it seems reasonable to call the protein "Koilin", if a specific designation is desired. It is, however, not certain that it represents a single substance, since the microscopic picture of the lining has been interpreted as if the strands of secretion emerging from the glands are glued together by "eine körnig strukturierte Zwischensubstanz, welche nicht in der inneren Hälfte des Drüsenschlauches, sondern in der Nähe seines Ausganges und in der zwischen den Drüsenschläuchen gelegenen Magenschleimhaut abgesondert wird" (STREESEMANN l. c., p. 162).

Since, however, the entire lining stains with eosin and with picric acid, the "Zwischensubstanz" and the rest of the lining must have certain properties in common, both of them being acidophilic.

[1] Already HEDENIUS (1892) had observed that the sulfur content of the gizzard lining is relatively low, and discussed the relation to the keratins on the basis of the data then available.

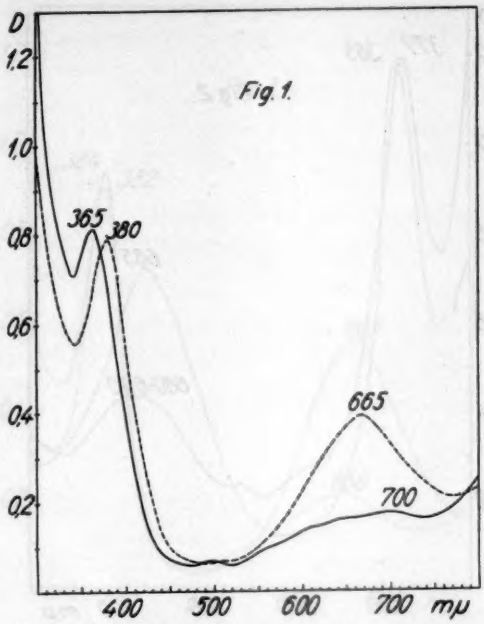

Fig. 1. Absorption curves for gizzard pigment extracted with acid methanol (———), and shaken down into chloroform (------).

Ordinate: Optical density.
Abscissa: Wavelength in mμ.

The pigment.

A. Chemical and spectroscopic characterization.

Most of the experiments described in this section were carried out on the defatted dried material described in the foregoing section. A few preliminary extraction experiments were made on the freshly separated lining.

Several methods of extracting the pigment were tried. Ether, chloroform, or water extracted very little of the pigment, if any. Hot methanol, ethanol, or acetone extracted some of the pigment with a greenish-yellow colour. By treatment with aqueous hydrochloric acid the lining changed its colour to blue or green according to the procedure used, but no extraction of pigment occurred.

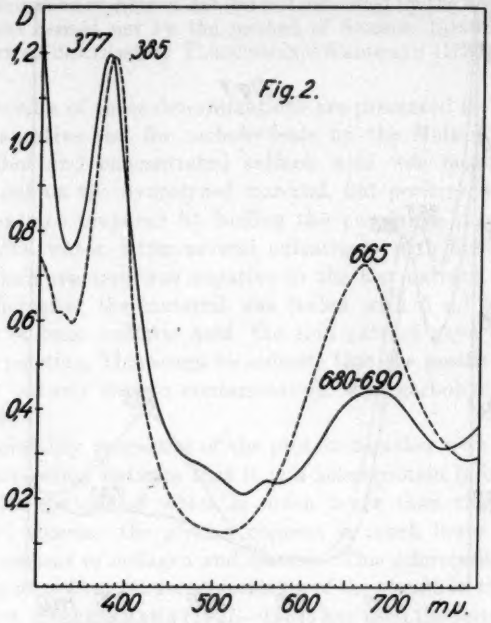

Fig. 2. Absorption curves for chicken bladder bile treated with acid methanol (———), and shaken down into chloroform (------).

Ordinate: Optical density.
Abscissa: Wavelength in mμ.

Heating with methanol plus concentrated hydrochloric acid (9 + 1 v/v) resulted in extraction of a large portion of the pigment with a blue-green colour. When water and chloroform were added to this solution, the blue-green colour passed into the chloroform layer.

On examination in the Beckman spectrophotometer, the impure methanolic solution showed an absorption maximum at 365 mμ and a somewhat flat absorption region in the neighbourhood of 700 mμ (Fig. 1). When the pigment was shaken down into chloroform, the absorption curve showed maxima at 380 mμ and 665 mμ (Fig. 1).

With 0.1 N aqueous NaOH or alkaline methanol nearly all the pigment could be extracted from the lining, in both cases with a

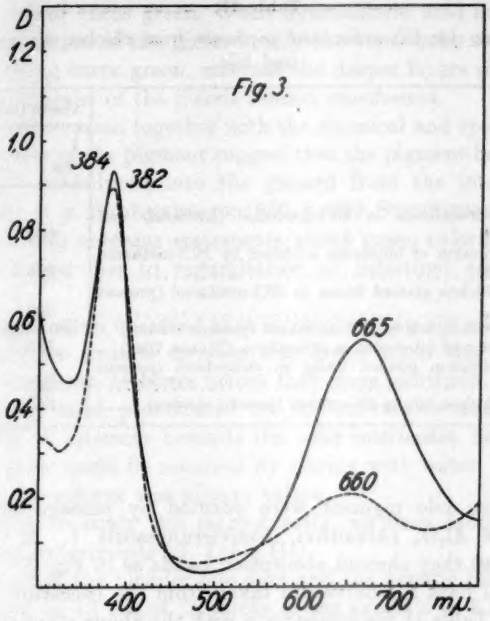

Fig. 3. Absorption curves for chloroform extracts of bile pigment (———) and of gizzard pigment (------), both after chromatography on Al_2O_3.

Ordinate: Optical density.
Abscissa: Wavelength in mμ.

yellow colour. When the yellow alkaline methanolic solution was acidified with concentrated HCl the colour changed into green. Subsequent alkalinization resulted in return of the yellow colour.

These observations are in accordance with the conception that the gizzard pigment is a yellow modification of biliverdin the main pigment of chicken bile.

The green pigment from chicken bladder bile could be extracted with chloroform after the bile had been diluted with acid methanol. The chloroform solution so obtained showed absorption maxima at 385 mμ and 665 mμ. Chicken bladder bile diluted with acidified methanol showed absorption maxima at 377 mμ and 680—690 mμ (Fig. 2).

When acid chloroform solutions of the gizzard pigment and of

Table II.

Spectral data for biliverdin and pigments from chicken gizzard lining and bile.

	Absorption maxima	
	I mμ	II mμ
Biliverdin hydrochloride in HCl-methanol (LEMBERG and LEGGE 1949)	680	377
Chloroform solution of biliverdin acidified by HCl-methanol (TIXIER 1945)	675	—
Pigment of chicken gizzard lining in HCl-methanol (present studies)	700	365
Pigment of chicken bile in HCl-methanol (present studies)	680—690	377
Methanolic ester of biliverdin in chloroform (TIXIER 1945)	665	384
Pigment of chicken gizzard lining in chloroform (present studies)	665	380
Pigment of chicken bile in chloroform (present studies)	665	385

the chicken bile pigment were purified by passage through a column of Al_2O_3 (Alkalifrei, Aktivierungsstufe 1., M. WOELM, ESCHWEGE) they showed absorption bands as in Fig. 3.

Spectral data for biliverdin taken from the literature are presented in Table II for comparison with the above mentioned data for chicken gizzard and bile pigments.

The Gmelin test with fuming nitric acid was positive when applied to a green solution of the gizzard pigment in chloroform as well as to a dry film of the residue obtained by evaporating the chloroform in a porcellain dish.

When the green residue from the chloroform solution of the pigment was dissolved in ammoniacal ethanol and zinc acetate and a few drops of iodine were added to it, the colour changed to blue-green with a red fluorescence in ultraviolet light. This behaviour is characteristic of bilatrienes (LEMBERG and LEGGE 1949), the group of bile pigments to which biliverdin belongs.

B. Location of the pigment in the stratum corneum and changes in the colour resulting from withdrawal of food and water with and without previous ligation of the bile ducts.

The pigment is concentrated in the ventricular surface of the lining. This is easy to observe on the detached lining, especially when the latter is treated with hydrochloric acid, whereby the

yellow colour turns green. When hydrochloric acid is applied to a frozen section of the gizzard, only the ventricular surface layer of the lining turns green, whereas the deeper layers of the lining and the content of the glands remain uncoloured.

This observation together with the chemical and spectral characterization of the pigment suggest that the pigment has its origin from bile introduced into the gizzard from the intestine. The literature (*e. g.* BUDDENBROCK 1956, p. 389; STREESEMANN, 1927—1934, p. 492) contains statements about green coloration of the gizzard lining due to regurgitation of intestinal content with bile.

We have often observed that in chicks of an age of more than 2 months the *stratum corneum* was green when the birds were fasted for about 24 hours before they were sacrificed. The green colour was most pronounced on the curvatures and increased gradually in intensity towards the *zona intermedia*. Some of the green colour could be removed by rinsing with water. The region around the *pylorus* was always yellow.

In order to study this more exactly, we have carried out the following experiments (cf. Table III).

Ligation of the bladder bile duct and the hepatic bile duct was performed on 13 out of 25 chicks of the age of 2 to 3 months receiving a commercial chicken ration (groups 3 and 4, Table III). Food and water were withdrawn from 6 of these chicks (group 3, Table III) immediately after the operation. They were killed 24 hours later. At the same time the 7 other operated chicks (group 4) were killed after having had access to food and water until the time of sacrifice.

Of the 12 unoperated chicks (groups 1 and 2), 6 (group 1) were without food and water for the last 24 hours before being sacrified, whereas the other 6 (group 2) had access to food and water until they were killed simultaneously with the others.

During the last hour before the chicks were killed, gastric juice was taken out from the gizzard by means of a plastic tube and a syringe. The colour of the juice was observed, and the pH determined by a semi-micro glass electrode adapted for use with 0.2 ml fluid. Autopsy was performed immediately after sacrifice, and the colour of the gizzard lining noted. The pH of the duodenal content was measured with the glass electrode in two regions, *viz.* near the pylorus and near the bile duct. Further, the colour was observed before and within 15 minutes after 5 N and 0.1 N HCl had been

Table

Data from the experiments

Group no.	Chick no.		Colour of gizzard lining	Gastric juice	
				pH	Colour[1]
1	5,581	.....	green	1.7	green
	5,555	.....	green	2.1	green
	5,556	fasting[3]	green	1.8	green
	5,594	.....	green	1.7	green
	5,601	.....	green	2.6	green
	5,490	.....	green	1.4	green
2	5,491	.....	yellow	1.4	slightly yellow
	5,597	.....	yellow	2.3	0
	5,598	.....	yellow	1.3	0
	5,599	.....	yellow	1.8	0
	5,600	.....	yellow	1.5	0
	5,603	.....	yellow	1.7	0
3	5,583		yellow	1.9	0
	5,582		yellow	2.0	0
	5,596	fasting[3], bile ducts ligated	yellow	1.9	0
	5,591		yellow	1.9	slightly yellow
	5,589		yellow	1.3	0
	5,587		yellow	2.3	0
4	5,584	.....	yellow	2.1	0
	5,586	.....	yellow	2.6	slightly yellow
	5,590	.....	yellow	1.5	0
	5,588	bile ducts ligated	yellow	2.1	0
	5,595	.....	yellow	1.8	0
	5,592	.....	yellow	2.2	0
	5,593	.....	yellow	1.7	0

[1] 0 means no appreciable colour.

[2] The colour of the duodenal contents near the pylorus and near the bile ducts was yellowish-brown in all instances. 0 means no distinguishable change of colour.

[3] Food and water deprived for 24 hours before sacrifice.

added to the duodenal contents from these two regions. pH of the bile was measured immediately after it had been removed from the bladder.

In all the unoperated chicks which had been fasted before sacrifice (group 1), the lining was green (with the exception of the region immediately around the pylorus). In the corresponding group of unoperated chicks (group 2) which had not been fasted, the lining had the normal yellow colour.

III.

described on pages 329—332.

Duodenal content						Bladder bile pH
near pylorus			near bile ducts			
pH	Colour[a] after addition of		pH	Colour[a] after addition of		
	5 N HCl	0.1 N HCl		5 N HCl	0.1 N HCl	
5.9	green	yellow	6.5	green	yellow	5.6
6.6	green	yellow	6.9	green	yellow	5.7
6.4	green	yellow	6.6	green	yellow	5.8
5.8	0	0	6.7	green	yellow	5.6
6.5	green	yellow	6.6	green	yellow	5.6
6.1	green	yellow	6.5	green	yellow	5.7
6.4	slightly green	0	6.3	green	yellow	6.7
6.2	0	0	6.1	0	0	6.3
6.3	0	0	6.2	0	0	6.4
5.0	0	0	6.0	0	0	6.2
6.1	0	0	6.2	slightly green	slightly yellow	7.1
6.1	0	0	6.3	slightly green	slightly yellow	6.8
6.2	0	0	6.4	0	0	7.7
6.6	0	0	6.5	0	0	7.7
6.4	0	0	6.0	0	0	7.7
5.1	0	0	5.7	0	0	6.4
6.6	0	0	7.1	0	0	7.8
6.4	0	0	6.5	0	0	7.7
6.4	0	0	5.9	0	0	7.5
6.2	0	0	6.2	0	0	7.9
5.9	0	0	6.0	0	0	7.8
6.2	0	0	6.4	0	0	7.6
6.3	0	0	6.3	0	0	7.7
6.5	0	0	6.4	0	0	7.8
6.2	0	0	6.2	0	0	7.8

The chicks which had their bile ducts ligated had yellow gizzard lining whether they had been fasted or not (groups 3 and 4).

This decisively shows that the green colour of the lining of the unoperated fasted chicks has originated from bile entering the gizzard through the pylorus. The chicks with green lining had also green gastric juice.

There was no significant difference in pH of the gastric juice

in the four groups. The same applies to the pH of the duodenal content.

The duodenal content was yellow-brownish in all 25 chicks. When 5 N HCl was added to the duodenal content of the unoperated fasted chicks (group 1), the colour of the content changed to green, whereas with 0.1 N HCl a change to yellow was found. There was one exception to these observations, *viz.* for chick no. 5594, where no change of colour occurred in the part of the *duodenum* which is near the pylorus.

Addition of 5 N, resp. 0.1 N HCl to the duodenal contents of the unoperated non-fasted chicks (group 2) resulted in colour changes in the same direction as in group 1 but less pronounced and only in half of the chicks.

In the chicks with bile duct ligature (groups 3 and 4), the colour of the duodenal content remained unchanged after addition of 5, resp. 0.1 N HCl, irrespective of whether the chicks had fasted or not.

The bladder bile of all 25 chicks was dark green. Its pH was markedly lower in the unoperated fasted chicks (group 1) than in the unoperated non-fasted (group 2). In the bladder bile of the operated chicks (groups 3 and 4) the pH was apparently uninfluenced by fasting (except for chick no. 5591) and not much different from that of unoperated non-fasted chicks. The bile bladders of the chicks with bile duct ligature were markedly dilated.

The main conclusions to be drawn from the data presented in Table III are 1: that the green colour of the gizzard lining observed after deprivation of food and water is dependent on the free outflow of bile into the intestine, and 2: independent of variations in the pH of the gastric juice collected by a stomach tube, as well as of the pH of the duodenal content. 3: The colour of the duodenal content (yellow-brownish) is not influenced by the outflow of bile into the duodenum, although 4: the duodenal content may become green with 5 N HCl and yellow with 0.1 N HCl only when bile has access to the intestine. 5: Access of the green bile to the duodenal content does not result in green colouration of the latter. 6: This shift of colour is not due to differences of pH values in the bile bladder and the duodenal content.

In chicks younger than one month, fasting did not, or at least less frequently, lead to green colouration of the lining.

C. Note on the development of the lining.

Several authors, *e. g.* CORNSELIUS (1925) have studied the *embryonic development* of the chicken gizzard.

In connection with the present chemical studies it might be of interest to add the following observations of our own on the development of the lining during the last weeks of incubation and the first few hours and days of extraoval life.

Macroscopically, in nine examined embryos of an age from 15 to 20 days the lining was soft and whitish. The content of the gizzard appeared as a white, gritty, soft "brei" of a pH ranging from 3.8 to 5.6. In 4 chicks up to five hours old the linings were slightly yellow, and the content of the gizzards appeared as a yellow, gritty, soft "brei", pH was 3.1 to 3.3. When 2.5 N HCl was added to the content the colour changed to green. The gizzard lining of 7 chicks of an age of from one to three days was yellow, and the colour changed to green after addition of 2.5 N HCl. The content of these gizzards was liquid and pH was 2.7 to 3.5. When the chicks were 3 to 4 weeks old, the pH of the gastric juice had fallen to about 2.0. This level was also found in chicks of an age of up to 13 weeks. Older chicks were not examined in this series.

Histologically, in the gizzard of a two-day old chick the lining appeared about three-fourths as thick as the glandular layer adjacent to it. The lining appeared eosinophilic and took a bright yellow stain with van Gieson's solution. In a one-hour old chick the lining stained slightly, but the substance in the *lumina* of the glands stained intensely with eosin and picric acid. In the embryos, both the content of the glands and the lining stained only slightly.

These results seem to indicate that the secretion of the tubular glands is not transformed into a horny substance until after hatching.

As a coincident observation it may be added that in the *proventriculus* of the one-hour and the two-day old chicks the multilobular glands almost completely filled the *tunica propria*, whereas in the embryos these glands occupied only one half to three-fourths of the *tunica propria*.

Summary.

1. The gizzard lining of chicks, 4 to 8 weeks old, was examined with respect to its protein and yellow pigment.

2. Seventeen amino acids were determined quantitatively. The protein which represents the secretion of the stratum glandulare is a scleroprotein having a lower content of cystine than most keratins and a lower glycine content than collagen and elastine.

3. The pigment was extracted with a mixture of methanol and hydrochloric acid, whereby it changed to a blue-green substance soluble in chloroform. Spectral and certain chemical properties of the extracted pigment characterize it as a bilatriene.

4. The pigment occurs in the ventricular surface of the lining. When a cross section of the gizzard is wetted with 2.5 N HCl and inspected under the dissection microscope, the surface of the lining gradually turns green. This green colour does not appear in the deeper layer or in the glands. Thus, the pigment must have originated from regurgitated intestinal content.

5. When chicks of an age of more than 2 months are deprived of food and water for 24 hours, the surface of the lining usually shows a green colour. If the hepatic and cystic bile ducts are ligated the green colour does not appear.

6. Data for colour and pH of bile, duodenal and gastric content under various experimental conditions are presented.

7. The yellow colour of the surface of the gizzard lining is observed already on the first day after hatching.

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Variations in Nucleic Acid Concentration during the Development of Early Chick Embryos.

By

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There are several recent investigations concerning the changes in nucleic acid concentration which occur during the development of the chick embryo. Here may be mentioned especially those by NOVIKOFF and POTTER (1948), REDDY, LOMBARDO and CERECEDO (1952) and LESLIE and DAVIDSON (1951).

It is a striking fact, however, that almost all authors dealing with the subject have confined their investigations to developmental stages older than 2 days. The most plausible explanation of this is certainly the difficulty in making a sharp distinction between embryonic and extraembryonic tissue in the early blastoderms, as it may be recalled that not until the third day of incubation is the embryo folded off from the underlying yolk, rendering the embryo body a well-defined part of the egg-contents.

In earlier stages the embryo is still in the same plane as the extraembryonic layers, and there is no sharp boundary between them and the embryo region. This fact is especially apparent in the very earliest stages before a primitive streak has yet developed.

Under such circumstances it is obviously very difficult to fix an unequivocal boundary line for the embryo proper within the early blastoderm. It is also significant that SOLOMON (1957), who has measured the desoxyribonucleic acid content of whole chick blastoderms, 18—70 hours old, does not make any attempt to distinguish different regions within the blastoderm until the 40-hour-stage.

However, by omitting all developmental stages younger than 2 days from the analyses, it is clear that an extremely important period in the development of the chick embryo, the gastrulation period, totally escapes observation. Generally one assumes that this period sets in almost immediately after the onset of incubation, and the process culminates with the full outgrowth of the primitive streak (at about the 20-hour-stage), which represents the fused lips of the blastopore.

In the present investigation the author has analyzed the concentration of desoxyribonucleic acid, ribonucleic acid and nucleotides in the embryo area of early blastoderms, covering the period from 0 to 60 hours of incubation. The amounts per cell of the nucleic acids, nucleotides and total-nitrogen have also been examined. Further the synthesis of desoxyribonucleic acid in embryo areas cultivated in vitro has been studied.

The author has found it most important to maintain a distinction within the blastoderm between one embryo region and one extraembryonal region for all stages investigated. As a matter of fact, it will be shown that the mass of the extraembryonal region strongly dominates that of the embryo region at all stages. It is obvious that in biochemical analyses comprising the whole blastoderm eventual variations, being confined to the latter region only, may easily be hidden or at least distorted.

Furthermore, the decision to concentrate the analyses upon a restricted embryo area of the dimensions stated in this investigation seems well justified, for the reason that such areas when cultivated in vitro on an adequate food supply will show approximately normal morphogenesis.

Material and Methods.

Material.

For the investigation I have used newly laid hen's eggs of pure breed (White Leghorn). The eggs have been obtained throughout the year

and no seasonal variations in the developmental ability of the embryos have been observed. As a rule the eggs have been dealt with immediately, but sometimes when necessary, storage has occurred, and then only for 7 days at the most at about 10° C.

The eggs have been incubated in a thermostatically controlled incubator at 37.5° C ± 0.5°, where they were placed out on netshelves. The atmospheric humidity in the incubator was maintained at a suitable level by keeping vessels of water in it. Under these conditions normal development was obtained for embryos of all stages.

Preparation of the Embryos.

At all stages the analyzed embryo is a quadrangular piece within the area pellucida of the blastoderm. In the present communication this central quadrangle is referred to as "embryo area". The longitudinal axis of this area coincides with the longitudinal axis of the developing embryo and its angles, except for the most advanced stages, touch the inner margin of the area opaca. In Fig. 1 some of the investigated stages are schematically outlined and respective embryo areas are marked off. As it appears from the figure, the "embryo area" here roughly corresponds to the "embryonal area" described in some textbooks, cf. PATTEN (1951).

For blastoderms of 0—6 hours of incubation the embryo area has the shape of a square. As it has not been possible to distinguish the embryo axis of such early blastoderms with absolute certainty, the square has been arbitrarily orientated within the area opaca. In spite of the fact that for these early stages the embryo area consequently will have a varying amount of such tissue material, which constitutes the embryo axis, the analyses are in good agreement. Attention may be called to the fact that for these stages every single analysis included at least 15 pooled embryo areas.

For blastoderms 6—12 hours old the embryo area still has the shape of a square, but from now on the longitudinal axis of the embryo can easily be distinguished.

For older blastoderms (14—60 hours) the embryo area always has a rectangular form.

Generally each separate analysis was made on the pooled material from 5—15 embryo areas. Special care was taken that only absolutely normally developed embryos were included. In the analyses the permitted difference of age among embryos representing one and the same stage of development has been about ± 1 hour, except for the earliest stages (0—14 hours) where a difference of ± 2 hours was allowed.

The classification of the different stages of development of the embryos is based upon the system of HAMBURGER and HAMILTON (1951).

The preparation of the blastoderms was carried out in 0.93 per cent NaCl. Before they were cut out from the yolk, they were first cautiously rinsed from below with saline, injected with a hypodermic syringe. As a rule the blastoderm adheres firmly to the membrana vitellina — especially in the beginning of incubation — and this mem-

brane can be used for holding the blastoderm in the subsequent preparation, which involves a thorough rinsing of the blastoderm in saline to remove the last of the adhering yolk from the embryo area. For embryos of 16—20 hours' incubation and older this was done comparatively easily, but for earlier embryos, which actually have many of their granules held by a loose reticulum of phagocytic cells, the procedure was arduous.

Among the adhering granules the white yolk granules were conspiciously many.

All the time it was necessary to proceed with great caution, as the blastoderms are very sensitive to mechanical injury.

When the blastoderms had been freed from the membrana vitellina, the embryo area was carefully dissected out and weighed on the diver balance, whereupon it was immediately put into the extraction vessel.

A satisfactory medium for the embryos during the preparations proved to be 0.23 per cent NaCl, and there has been no reason to exchange it for chicken-ringer, except when cultivation in vitro was planned. The temperature of the saline was held at about 20° C.

Before extraction the embryo areas or parts of them were immersed in saline for varying periods of time, but in no case has the interval from opening the egg until putting the embryo area (total or divided) into the extraction vessel exceeded 15 minutes.

Preparation of Embryos under Sterile Conditions.

After rubbing the eggshell with a pellet of cotton-wool soaked in 70 per cent ethanol, the shell was broken, the white was removed, and the yolk wasput into sterile chicken-Ringer of the following composition:

Minimum glucose medium according to SPRATT (1948), modified according to HOWARD (1953).

NaCl	0.650 per cent
KCl	0.033
$CaCl_2 \cdot 6\ H_2O$	0.031
$Na_2HPO_4 \cdot 2\ H_2O$	0.007
KH_2PO_4	0.003
$NaHCO_3$	0.055
Glucose	0.850

With sterile scissors and forceps the blastoderm was then taken out of the yolk, and adhering granules were removed by gently sucking the dissected embryos back and forth in a sterile pipette. After the weighing the control embryos were immediately put into the extraction vessel for estimation of their content of nucleic acids; the others were washed two times in sterile chicken-Ringer and were then transferred into Carrel-flasks on a medium of the same composition as the chicken-Ringer with the addition of 0.42 per cent agar.

It appeared that special care had to be taken that the chicken-Ringer

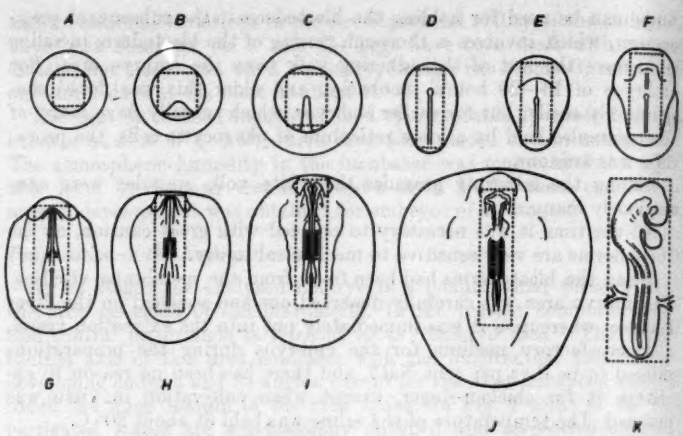


Fig. 1. Schematic drawings of some early developmental stages of the chick embryo. Embryos incubated for the following number of hours are represented: A: 0, B: 6, C: 12, D: 18, E: 20, F: 23, G: 26, H: 30, I: 34, J: 40, K: 60. In D—K only the central region of the blastoderm is included. Dotted lines indicate the dimensions of the embryo area.

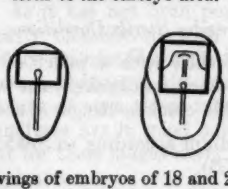


Fig. 2. Schematic drawings of embryos of 18 and 23 hours of incubation. Heavy lines limit the part of the embryo which is cultivated in vitro.

which had been brought into the flasks together with the embryos was carefully sucked off from the stiffened agar-substratum before closing the flasks. Otherwise normal morphogenesis would not take place: the embryos floated upon the liquid and assumed a globular form.

The Carrel-flasks were held in an incubator for exactly 24 hours at 37.5° C $\pm$ 0.5°. After this incubation the embryos were taken out of the flasks, morphogenesis was controlled, and the embryos after weighing were immediately placed into the extraction vessels for analysis on nucleic acids.

Preliminary observations had shown, however, that morphogenetic changes of embryos cultivated in vitro were easier to observe if the explanted embryo did not include the whole embryo area, as delineated in Fig. 1, but only the anterior half of it. Accordingly only that part of the embryo area was used in all the in vitro cultivations, and the data given for cultivated embryos and their controls refer only to that region.

In all cases Hensen's node was included in the explanted part of the embryo area. In Fig. 2 is shown the extension of this part for blastoderms of 18 resp. 23 hours of incubation.

Determination of the Mass of the Embryos.

In most investigations on chick embryos the concentration of different chemical substances in the embryo body is referred to wet weight. Such a reference can be accepted for embryos older than 2 days, but for younger embryos the value of it can be seriously doubted. Obviously the dry weight will in the latter case give a more reliable expression for the embryos' mass.

I have found it most advantageous in all cases to determine the mass of the embryos by using the Cartesian diver balance, ZEUTHEN (1948), in which embryos are weighed with very high accuracy submersed in saline or chicken-Ringer.

With this method the weight of the embryos will be expressed as "reduced weight" (R. W.), which is the total weight of the object considered minus the weight of the liquid which is displaced by the object.

The diver used for the purpose was made of glass with a collodion cup on top. With the exception of some embryos cultivated in vitro and their controls, one and the same diver has been used for all the embryos investigated.

As reference weights for the embryos small polystyrene beads have served. In 0.93 per cent NaCl a standard bead of the absolute weight 2.146 mg and the specific gravity 1.064 was exactly counterbalanced by applying a subpressure of 30.5 $\pm$ 0.2 cm water in the manometer.

The relative weight of the embryos (R. W.$_x$) is calculated from the following formula:

$$\text{R. W.}_x = \text{R. W.}_{st} \times \frac{1 - \dfrac{B}{B - p_x}}{1 - \dfrac{B}{B - p_{st}}}$$

R. W.$_{st}$ is the relative weight of the reference weight, B represents barometric pressure which may be put to 1,000 cm H_2O, p_x and p_{st} stand for changes in equilibrium pressure of the system resulting from loading the diver with embryo or standard weight. From the formula it is obvious that no proportionality exists between R. W.$_x$ and p_x. In my case simple calculus has shown that the difference from this proportionality will be very small, however, and may in fact be neglected, especially as the loads in the different weighings have as far as possible been adjusted to be fairly alike. Accordingly the relative weight can be expressed as p_x, that is in cm water, with an accuracy of $\pm$ 0.2 cm.

In the weighings it was necessary to exercise great caution that no small air bubbles, which could act as floats, adhered to the embryos.

Experiments had shown that the adhering yolk granules had a specific gravity nearly corresponding to that of the embryo tissue and widely differing from the specific gravity of the media used.

In this investigation no special efforts have been taken to obtain the absolute weight of the embryos, as for a comparison between embryos of different stages of development information about the relative weight will be quite sufficient.

It was obvious, however, that it would be advisable to have an additional, more common standard than R. W. to refer the obtained biochemical data to. For that reason analyses of total-nitrogen in series of blastoderms, embryos and parts of embryos were performed according to the method of BOISSONNAS and HASELBACH (1953). In this method all nitrogen is first converted into ammonia as in the ordinary micro-Kjeldahl method. After addition of a ninhydrin-hydrindantin reagent, a blue NH_3-complex is formed by heating and is then colorimetrically estimated. Standard deviation $\pm$ 2 per cent. As reference a very dilute solution of $(NH_4)_2SO_4$ was used. For the readings a Klett-Summerson photoelectric colorimeter was employed.

Determination of Nucleic Acids and Nucleotides.

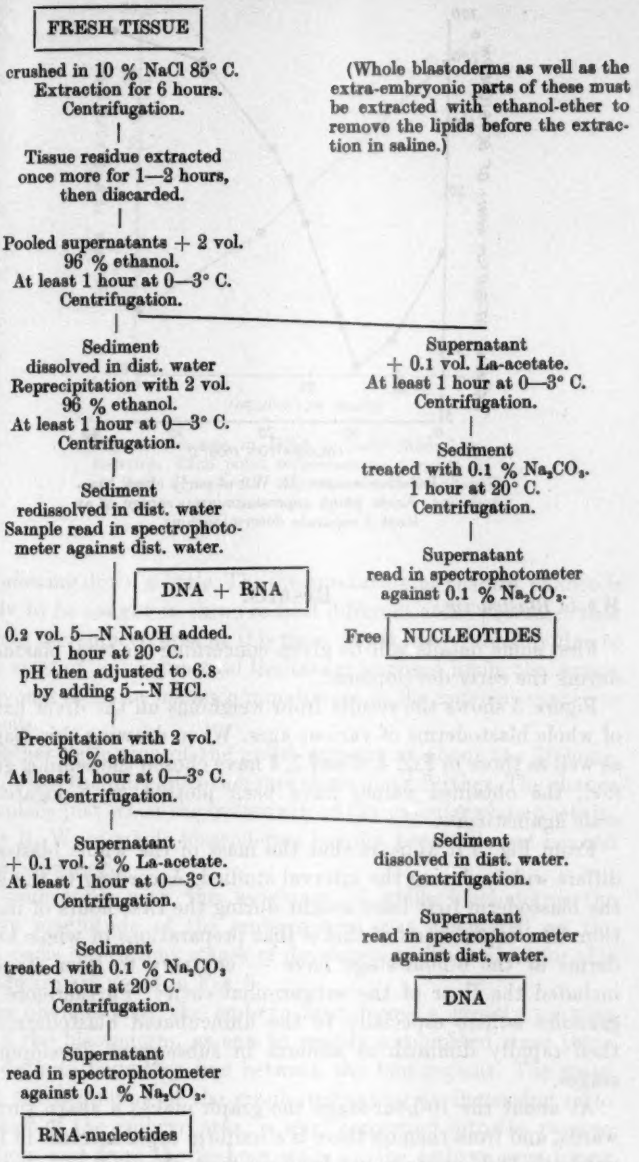
The principal method used has been that of BENDICH (1952) as modified by LINDH (1956). When originally proposed by BENDICH, the method was intended for preparative work, but it has proved very suitable for analytical purposes too. A plan of the steps in the method is given on next page.

When analyzing moderate amounts of embryos it has been necessary to work with small volumes of liquid, so, for instance, the total volume of the extraction liquid seldom exceeded 0.5 ml.

For the quantitative estimation of the nucleic acids and nucleotides the values for absorption at 260 mμ have been used, this being the position for the absorption-maximum in the curves. The absorption-minimum of the nucleic acids has generally been at 230 mμ, but sometimes it has shifted to 235—240 mμ, presumably owing to contamination with protein. The readings have extended over the interval 230—280 mμ. The absolute amount of nucleic acid and nucleotides in the samples has been calculated from comparison with standard solutions made up from commercial preparations of ribonucleic acid.

For the spectrophotometer-readings a Hilger Uvispek spectrophotometer has been used. Thickness of absorbing solution: 10 mm. Volume of sample: 0.30—0.40 ml.

The results obtained with the method now described have been in good agreement with results obtained for the same material with the Ogur-Rosen method and with the Schmidt-Thannhauser-Schneider method. Furthermore, chromatographic analyses of the RNA-hydrolysate and of the DNA-fraction hydrolyzed in formic acid confirm the reliability of the method.


FRESH TISSUE
crushed in 10 % NaCl 85° C.
Extraction for 6 hours.
Centrifugation.
Tissue residue extracted once more for 1—2 hours, then discarded.
Pooled supernatants + 2 vol. 96 % ethanol.
At least 1 hour at 0—3° C.
Centrifugation.
(Whole blastoderms as well as the extra-embryonic parts of these must be extracted with ethanol-ether to remove the lipids before the extraction in saline.)
Sediment
dissolved in dist. water
Reprecipitation with 2 vol. 96 % ethanol.
At least 1 hour at 0—3° C.
Centrifugation.
Sediment
redissolved in dist. water
Sample read in spectrophotometer against dist. water.
DNA + RNA
0.2 vol. 5—N NaOH added.
1 hour at 20° C.
pH then adjusted to 6.8 by adding 5—N HCl.
Precipitation with 2 vol. 96 % ethanol.
At least 1 hour at 0—3° C.
Centrifugation.
Supernatant
+ 0.1 vol. 2 % La-acetate.
At least 1 hour at 0—3° C.
Centrifugation.
Sediment
treated with 0.1 % Na_2CO_3
1 hour at 20° C.
Centrifugation.
Supernatant
read in spectrophotometer against 0.1 % Na_2CO_3.
RNA-nucleotides
Sediment
dissolved in dist. water.
Centrifugation.
Supernatant
read in spectrophotometer against dist. water.
DNA
Supernatant
+ 0.1 vol. La-acetate.
At least 1 hour at 0—3° C.
Centrifugation.
Sediment
treated with 0.1 % Na_2CO_3.
1 hour at 20° C.
Centrifugation.
Supernatant
read in spectrophotometer against 0.1 % Na_2CO_3.
Free NUCLEOTIDES

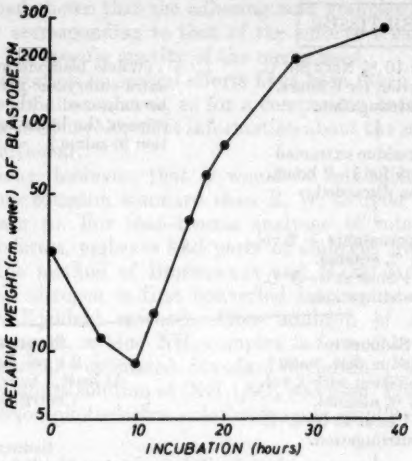


Fig. 3. Relative weight (R. W.) of early chick blastoderms. Each point represents an average of at least 3 separate determinations.

Results.

Whole Blastoderm.

First some details will be given concerning the total blastoderm during the early development.

Figure 3 shows the results from weighings on the diver balance of whole blastoderms of various ages. When drawing this diagram as well as those in Fig. 4, 6 and 7, I have chosen the semilog graph, *i. e.*, the obtained values have been plotted on a logarithmic scale against time.

From Fig. 3 it appears that the mass of the whole blastoderm differs widely during the interval studied. According to the graph the blastoderm first loses weight during the first hours of incubation. The explanation of this is that preparations of whole blastoderms at the 0-hour-stage have — contrary to later stages — included the floor of the subgerminal cavity. Furthermore yolk granules adhere especially to the unincubated blastoderms and then rapidly diminish in amount in subsequent developmental stages.

At about the 10-hour-stage the graph makes a sharp turn upwards, and from then on there is a uniform rapid increase in R. W. until the 18-hour-stage has been reached, the multiplication rate

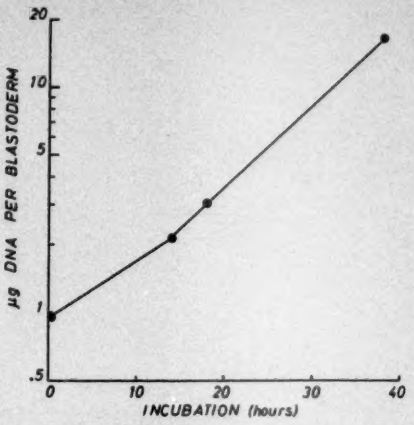


Fig. 4. Amount of DNA in early chick blastoderms. Each point represents an average of at least 3 separate determinations. Standard deviation max. ± 10 %.

then slowing down a little. The interpretation of the last change is hardly to be sought in those radical differentiation processes that begin in the embryo area at this time, but is more probably due to some sort of environmental limitation imposed upon the extra-embryonal cells, for instance impairment in the nutrient supply to the cells.

Another deflection of the graph appears at about the 28-hour-stage, when the multiplication rate slows down further. The change takes place just when the outgrowth of the vascular system sets in.

The R. W. of whole blastoderms has not been followed beyond the 48-hour-stage.

In connection with the weighings of whole blastoderms the relative magnitude of the embryo area was calculated on the R. W.-basis for various stages of development. The values for this relationship are given in Fig. 5.

It is obvious that the embryo area forms a greatly varying part of the blastoderm, as can be readily anticipated since there is no definite boundary line between the two regions. The steep rise in the beginning of the graph, indicating an increasing ratio on behalf of the embryo area, is soon converted into the reverse condition, and from the 20-hour-stage on the embryo area forms

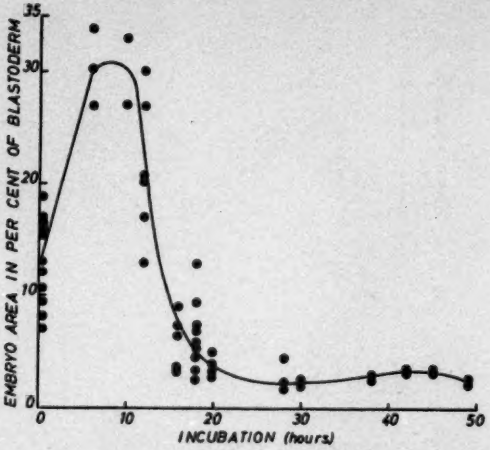


Fig. 5. Relative mass of the embryo area in per cent of R. W. of the whole blastoderm. Each point represents 1 determination.

only about 3 per cent of the whole blastoderm. The graph suggests that there is an exceptional increase in the relative mass of the extraembryonal area taking place in blastoderms of between 10 and 20 hours of incubation.

A further indication of a changed rate of growth in blastoderms of this age is given in Fig. 4, which shows the DNA content of whole blastoderms of different ages. The graph reveals a small deflection, indicating a changed rate of synthesis of the DNA. Owing to the small number of stages represented in the graph, the exact position of the deflection cannot be localized, but it seems probable that the accelerated rate of DNA-synthesis is initiated about the 14-hour-stage.

From Fig. 4 it will be seen that during the first 24 hours of development the total amount of DNA in whole blastoderms increases from 0.95 μg to 5.15 μg, that is a net synthesis of 4.20 μg. For the interval between 14 and 38 hours a doubling of existing DNA is achieved in 8.0 hours.

Embryo Area.

In Fig. 6 are collected data for the amounts of nucleic acids and nucleotides per unit of R. W. (cm water). The graphs show con-

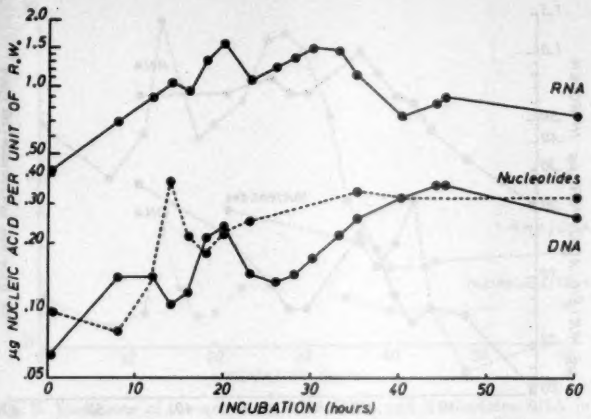


Fig. 6. Variations in concentrations of DNA, RNA and nucleotides in the embryo area during development, as evaluated per unit R. W. Each point represents an average of at least 5 separate determinations. Standard deviation max. ± 10 %.

siderably varying courses with several maxima and minima, localized at the following hours of incubation:

	Maxima	Minima
DNA	8, 20, 45	14, 26
RNA	14, 20, 30, 45	23, 40
Free nucleotides	14, 35	8, 18

In the graphs there appears a close inverse relation between DNA and the free nucleotides. Such a relation can also be traced between RNA and DNA, even if it is not so pronounced.

In order to complete the above determinations of the varying amounts of nucleic acids and nucleotides per R. W., the relation between them and total nitrogen content has also been investigated. In this case nitrogen content may be considered as an approximate measure of protein content. The application of such a common standard of reference as nitrogen content has also the advantage that it facilitates a comparison with results from other authors.

In Fig. 7 are given the variations in DNA, RNA and nucleotides calculated on the basis of total nitrogen content. Accordingly the values also include the nitrogen which is contained in the nucleic acids and the nucleotides themselves (about 12 per cent).

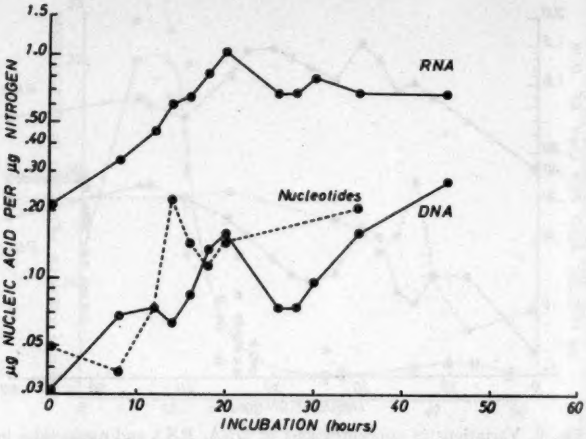


Fig. 7. Variations in concentrations of DNA, RNA and nucleotides in the embryo area during development, as evaluated per unit of nitrogen. Each point represents an average of at least 5 separate determinations. Standard deviation max. ± 10 %.

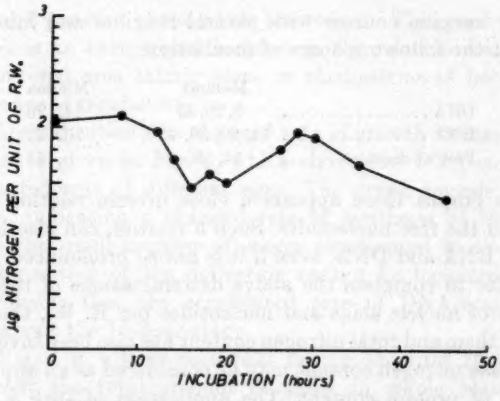


Fig. 8. Variations of the quotient R. W./Nitrogen in the embryo area during development.

When comparing the graphs in Fig. 6 with those in Fig. 7 it will be realized, however, that no fundamental changes have been produced by this new way of dealing with the subject. Principally the changes are restricted to the RNA graph.

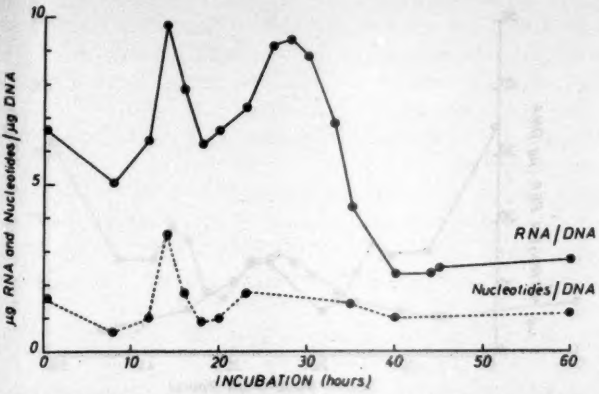


Fig. 9. Variations of the quotients RNA/DNA and Nucleotides/DNA in the embryo area during development. The amounts per cell (in µg) of RNA and nucleotides are obtained from multiplying the above values with 2.6×10^{-6}.

In this connection it may also be of interest to follow the variations in total nitrogen content as calculated per unit of R. W. The values are given in Fig. 8. The general impression is that there is a good correlation between R. W. and nitrogen content in the embryo area.

After having scrutinized the results so far one cannot avoid considering the question of whether the observed variations are really indicative of contemporaneous transformations in the individual cells, *i. e.*, if the changes in the cells are of the same kind and of the same dimensions as in the diagrams above.

Since neither of the two standards of reference which have been applied, R. W. and nitrogen content, seems to be free from variations itself, we cannot expect them to give us completely reliable information in this respect.

For a fully satisfactory answer to the question a direct analysis of isolated cells is of course necessary, but such an undertaking would require very expensive and complicated equipment.

However another simpler solution to problems of this sort has been suggested by DAVIDSON and LESLIE (1950). Their method does not yield information about any single cells, but nevertheless we shall obtain valuable information about average conditions for the whole cell population.

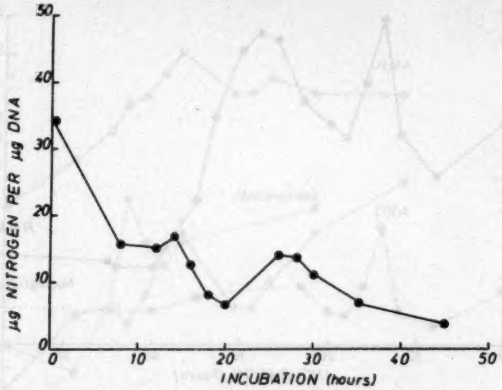


Fig. 10. Variations of the quotient Nitrogen/DNA in the embryo area during development. The amount per cell (in µg) of nitrogen is obtained from multiplying the above values with 2.6×10^{-6}.

The method in question is based upon the assumption of a constant amount of DNA in all the somatic cells of an animal organism — though varying for different species — a theory, which is now widely accepted. On the presumption that the investigated cell material is relatively homogeneous, the true course of biochemical variations in the average cell will according to DAVIDSON and LESLIE become apparent from simply putting the various biochemical data for a tissue portion in relation to its simultaneously estimated content of DNA, *i. e.*, by using DNA as the standard of reference. The experiments presented by the above authors were performed upon in vitro cultivated fragments from chick embryos more than 8 days old. The results were based upon an estimated amount per cell (nucleus) of $2.3—2.6 \times 10^{-6}$ µg DNA.

For the application in the present case of the method just mentioned only the earliest embryo areas have been considered tolerably homogeneous with reference to their cell population. After 24 hours of incubation the differentiation processes in the embryo area have proceeded so rapidly and are of such dimensions, that "average cells" in the proper sense of the term can no longer be considered. Even when dealing with the earliest embryo areas in this new manner, it must be borne in mind, how-

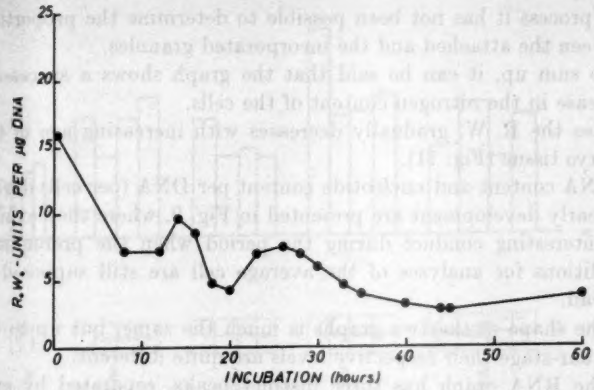


Fig. 11. Variations of the quotient R. W./DNA in the embryo area during development.

ever, that for small restricted parts of the investigated area variations may actually occur without being discovered.

In figures 9, 10 and 11 the data for RNA, nucleotides, nitrogen content and R. W. are arranged as proposed.

If we first pay attention to the standards of reference which have been applied earlier, nitrogen content and R. W., we can expect to receive direct information as to how constant they really have been during the period in question. It appears now that there have actually been great variations in both of them.

In the graph demonstrating nitrogen content per cell (figure 10) the contamination from yolk granules, which has been mentioned now and then, appears more distinctly than before. Thus the nitrogen content is shown to be of quite a different magnitude for the 0-hour-stage than for subsequent stages. Already at the 8-hour-stage it has been reduced to half the original size. Consequently there is scarcely any doubt that the figure for the 0-hour-stage is somewhat misleading, due to an excessive adherence of yolk granules. Notwithstanding it is probably still correct to regard the 0-hour-stage as having a relatively high nitrogen content. The reason for this opinion is that a thorough inspection of fixed and stained blastoderms revealed that part of the yolk granules, which at lower magnification have seemed to be superficially attached to the tissue, actually are incorporated into the tissue cells. On account of the losses of granules during the fixa-

tion process it has not been possible to determine the proportion between the attached and the incorporated granules.

To sum up, it can be said that the graph shows a successive decrease in the nitrogen content of the cells.

Also the R. W. gradually decreases with increasing age of the embryo tissue (Fig. 11).

RNA content and nucleotide content per DNA (per cell) during the early development are presented in Fig. 9, where they exhibit an interesting conduct during the period when the prerequisite conditions for analyses of the average cell are still supposed to prevail.

The shape of the two graphs is much the same, but up to the 40-hour-stage their respective levels are quite different.

The RNA graph has three distinct peaks, separated by even intervals. It is of special interest that the first of these peaks has its position at the 0-hour-stage, a fact that scarcely can be ascribed to adhering yolk granules. The other peaks hold the same positions as they did when R. W. and nitrogen content served as the standards of reference, *i. e.*, at 14 and 26 hours of incubation.

In the present investigation the quotient RNA/DNA has been further made use of also for investigating the regionally varying conditions in single embryo areas.

For this purpose embryos of various ages were divided into pieces, generally 4 in number, and each of these was then analyzed for DNA and RNA. Usually the analyses were performed so that corresponding pieces from embryos of exactly the same size and development were brought together and the estimation was then made on the pooled material. Control weighings of the separate pieces on the diver balance made it possible to obtain a uniform material.

The results are demonstrated in Fig. 12, where to the left the full dimensions of the investigated embryo area are given together with the exact position of the partition lines. To the right the values for separate pieces are arranged as horizontal stems at the same level as the pieces in question. In spite of the division of the embryo in separate regions, the principal RNA variations per DNA for the whole embryo area — already dealt with in connection with Fig. 9 — can still be detected here.

From these embryo profiles it appears that the quotient RNA/DNA can attain fairly different values within one and the same embryo. This is, however, by no means a random variation, on

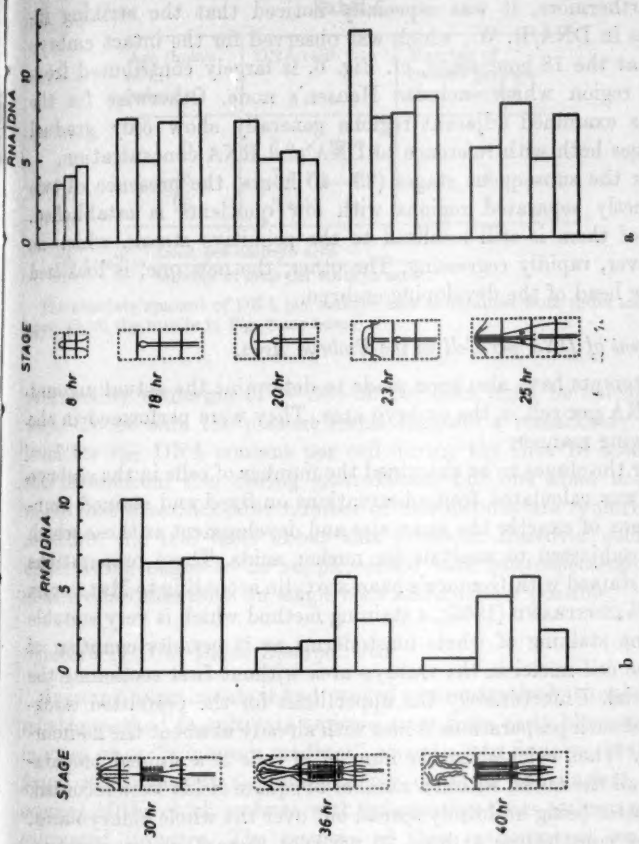


Fig. 12. Regional differences in size of the quotient RNA/DNA for early developmental stages of the chick embryo. To the left the investigated embryo area is outlined with the partition lines marked off. Opposite to the right the respective quotient is given in the shape of a horizontal stem. Average values of 3 separate determinations.

the contrary the regional differences can be traced back to already known facts concerning metabolism in the early chick embryo.

A close inspection of the diagrams will show that the four younger stages (8—23 hours) contain one particular region with a quotient which is distinctly lower than those of the adjacent regions, and as a matter of fact, even lower than for any other region. In all cases this region can be traced to that part of the embryo area which includes Hensen's node, *i. e.*, the foremost part of the primitive streak.

Furthermore, it was especially noticed that the striking increase in DNA/R. W., which was observed for the intact embryo area at the 18-hour-stage, cf. Fig. 6, is largely contributed from that region which includes Hensen's node. Otherwise for the stages examined adjacent regions generally show only gradual changes both with reference to DNA and RNA concentration.

For the subsequent stages (25—40 hours) the presence of two distinctly separated regions with low quotients is established. One of them is still localized to the primitive streak, which is, however, rapidly regressing. The other, the new one, is localized to the head of the developing embryo.

Amount of DNA per Cell in the Embryo Area.

Attempts have also been made to determine the actual amount of DNA per cell in the embryo area. They were performed in the following manner:

For the stages to be examined the number of cells in the embryo area was calculated from observations on fixed and stained preparations of exactly the same size and development as those which were subjected to analysis for nucleic acids. These preparations were stained with Gomory's haematoxylin according to MELANDER and WINGSTRAND (1953), a staining method which is very suitable for the staining of whole blastoderms as it permits counting of all the cell nuclei in the embryo area without first sectioning the material. Unfortunately the upper limit for the permitted thickness of such preparations is met with already at about the 26-hour-stage. When calculating the number of cells in a stained preparation, all the nuclei within a number of square fields were recorded, the latter being uniformly spread out over the whole embryo area, totally constituting at least 25 per cent thereof. Before extrapolating the total amount of cells in the embryo area, it was first checked that the interspace did not show a density of cells which markedly differed from that in adjacent fields actually counted over.

Until now only a small number of registrations have been performed. The result is given in Table I.

For comparison it may be recalled that DAVIDSON and LESLIE (1950) as well as HERRMANN (1952) have calculated with 2.6×10^{-12} g as the DNA content of cells (nuclei) in early chick embryos.

Obviously the figures presented are fairly uncertain and

Table I.

Age (hours of incubation)	DNA/cell[1]	Number of stained preparations counted
0	7.0×10^{-12} g	2
18	$7.1 \times$ »	2
26	$3.6 \times$ »	2

[1] DNA/cell = $\frac{\text{DNA per embryo area}}{\text{number of cells per embryo area.}}$

The absolute amount of DNA per embryo area is obtained from those analyses upon which the results in Fig. 6 are based.

presumably a margin of ± 30—50 per cent must be calculated with. To be sure the present result suggests a remarkably high level for the DNA content per cell during the first 18 hours of the incubation, *i. e.* during gastrulation, but one must bear in mind that a considerable number of calculations are required to ascertain the real facts about this problem. Moreover such an undertaking ought to be supplemented with microspectrophotometric determinations on single cells and nuclei if possible.

Synthesis of DNA in the Embryo Area.

As in my experiments it had proved a comparatively simple and reliable method to cultivate embryo areas from early blastoderms in vitro on a "minimum medium" according to SPRATT (1948), I found it convenient to complete my data for the nucleic acid content of the chick embryo with information from embryo areas cultivated in vitro. The purpose of these experiments was to determine whether there is a real new synthesis of DNA in the embryo area at early stages of the development of the chick embryo.

In these experiments the embryo areas accordingly may be considered as separate organisms, each forming an isolated, well defined system shut off from all the stored nutrients in the egg. SPRATT has already demonstrated that in early chick embryos developing on the medium referred to, cell differentiation and morphogenesis will continue at approximately the same rate as in controls developing in the intact egg. Of course explanted embryos become smaller than the latter, as growth in the sense

Table II.

Age of embryos in hours	Embryos cultivated in Carrel flasks		Control embryos not cultivated		Per cent increase of DNA/R. W. for embryos cultivated in Carrel flasks
	Number of samples[1]	DNA/R. W.[2] $\mu g \times 10^3$	Number of samples[1]	DNA/R. W. $\mu g \times 10^3$	
14—16	3	6.36 ± 1.0	3	2.62 ± 0.03	140
18	2	11.29 ± 0.94	2	8.47 ± 0.77	33
18—20	9	23.40 ± 2.48	8	16.00 ± 1.45	46
23	3	16.47 ± 4.42	3	6.11 ± 0.97	170
29	1	11.05	1	7.45	48

[1] Each comprising 5—18 embryo areas. Cf. Fig. 2.

[2] DNA/R. W. for the explanted embryos $= \frac{\text{amount of DNA after cultivation}}{\text{R. W. before onset of cultivation}}$

As the DNA content per unit of R. W. has proved to vary only slightly for uncultivated embryo areas of the same size and development it can be taken for granted that for the explanted embryos the quantity

$$= \frac{\text{amount of DNA before onset of cultivation}}{\text{R. W. before onset of cultivation}}$$

is directly identical with DNA/R. W. of the corresponding controls.

The age of the embryos refer to their stage of development at the beginning of cultivation.

of protein incorporation is out of the question, owing to lack of proteins or protein derivatives in the medium.

The results of my experiments are demonstrated in Table II.

The experiments clearly prove the existence of a synthesis of DNA in the explants, even if this synthesis is of greatly varying size. Such a result was also to be expected, as histologic examination of embryo areas cultivated in vitro had revealed the occurrence of a high mitotic activity there. However in the latter case, the possibility could not be excluded that the embryos were entirely dependent on DNA which in some way was stored in the cells of the early embryo.

Besides the DNA the experiments have also provided information about RNA and free nucleotides.

For RNA the general characteristic is a lower concentration in the explants than in controls. This finding obviously suggests the possibility of a conversion of RNA into DNA.

Also in explants the nucleotide fraction displays an inverse relation to the DNA. Consequently in explants with high DNA concentration the nucleotide concentration is low and vice versa.

Discussion.

Attention has already been called to the inverse relation between DNA and RNA in Fig. 6 and 7.

Although there is one simultaneous maximum for both of them — at 20 hours of incubation — the corresponding behaviour of the nucleic acids at this stage gives one the impression that the noticed effect may rather be ascribed to an abrupt decrease in the R. W. of the embryo. Such an opinion is also supported from the graph in Fig. 10, which indicates a rapidly diminishing proportion of nitrogen in the embryo tissue at that time.

This inverse relation between DNA and RNA as shown in the graphs is of great interest, bearing in mind the fact that the hen's egg forms a closed system. As was to be expected, this relation becomes still more apparent when the analyses are restricted to the isolated embryo areas cultivated in vitro. In the latter case there are in fact strong reasons in favour of a conversion of RNA into DNA.

AGRELL (1952) similarly found an inverse relation between the nucleic acids in insect pupae (Calliphora) during metamorphosis. Here too the investigated material represented a closed system.

In the graphs in Fig. 6 and 7 a decrease in the amount of DNA per unit of mass, connected with a simultaneous increase in the amount of RNA, may be interpreted as a slight arrest in cell multiplication with predominance of protein synthesis and cell differentiation. This view is based upon the generally recognized idea of there being a close connection between RNA concentration and protein synthesis. When examining Fig. 9, demonstrating the amount of RNA per unit of DNA (per cell), it is moreover found that the accumulation of RNA at 14 and 26 hours of incubation respectively is even more pronounced here than it appeared in Fig. 6 and 7.

As to the intervening periods, *i. e.*, those which in Fig. 9 are characterized by a low RNA/DNA quotient, it seems in the present situation natural to suppose that they represent conditions of intense cell multiplication.

From the diagrams above there will thus appear the following general picture of the processes taking place in the embryo: At the beginning of the incubation the embryo cells, which after the laying of the egg have been arrested in their activity until

now, start a general multiplication, culminating after about 10 hours of incubation. After this "wave" there follows a period with differentiation processes predominating in the embryo area, this condition being most pronounced at the 14-hour-stage. In turn it is superseded by a second "wave" of cell multiplication at about the 20-hour-stage. At about the 25-hour-stage there is another period of intensified protein synthesis, and finally a third "wave" of cell multiplication follows at the 35-hour-stage.

However one must not imagine an extreme dominance of either of the two processes, cell multiplication and cell differentiation, during the period investigated. Instead it may be considered as only a temporary intensification of the respective process.

This proposed scheme for the developmental processes in the embryo area is also supported by the observation that some experiments with the obvious purpose of attaining a synchronization of cell divisions in chick embryo cells, EMANUELSSON (1957) have revealed that for cells from the primitive streak stage the total time for mitosis + interphase will be 10—12 hours, *i. e.*, the same value as for the interval between the suggested maxima for cell multiplication above.

Moreover it appeared from preliminary observations of the mitotic activity of the embryo area that for the period of development between 16 and 26 hours the mitotic activity has a maximum at the 20-hour-stage.

Furthermore there is reason also to deal in this connection with the DNA synthesis of the embryos cultivated in vitro. As previously remarked the dimensions of the observed increase of DNA varied among the different developmental stages. Of course one must not try to draw too far-reaching conclusions from these variations, but they can easily be made to tally with the conditions already stated.

As suggested above, embryos explanted at about the 15-hour-stage are probably just preparing for an intense cell division, and consequently it may be expected that they will complete the DNA synthesis involved. On the other hand, embryos explanted at the 20-hour-stage are taken just when the suggested cell multiplication has reached a maximum in the embryo area, and accordingly a rich supply of nutrients is necessary for the cells to enable them to continue their synthesizing processes. When provided with only the very simple glucose medium used here, their capacity

for synthesizing DNA among other compounds is markedly lowered.

It was to be equally expected that embryos of 23 hours of incubation should be characterized by an intense DNA synthesis, which would again decrease when the embryo had reached the 29-hour-stage.

The stated regularity in the variations of the nucleic acids and nucleotides, as well as the additional facts connected with the matter, give one the impression that there exists a certain synchronism in activity among the cells in the early embryo area. However this will be soon disrupted by the rapidly progressing differentiation, and it is obvious that the appearance of regional morphogenetic processes in the embryo area can hardly be associated with any appreciable synchronism among the cells.

Possibly the observed conditions are directly concomitant with the synchronism existing among the embryo cells during the first developmental stages, before the egg has yet left the hen's body.

At the present stage of the investigation the results do not permit a definite statement regarding the question of whether there also exist variations in concentration of RNA and DNA in the area opaca, corresponding to those in the embryo area.

When comparing the general conduct of the nucleic acids for the investigated period with conditions prevailing during later periods in the development of the chick embryo, LESLIE (1955), some striking differences can be demonstrated.

The figures referred to prove that the general tendency for changes in the DNA content during development of the embryo is a decrease in concentration, both when the latter is evaluated per unit of weight and per unit of nitrogen. From this information one can draw the conclusion that for the major part of the development of the chick embryo, cell size and protein content per cell steadily increase.

However during the early period dealt with in the present investigation, the opposite feature is met with *i. e.* a definite increase in DNA concentration is instead obtained when calculating the amount of DNA per unit of R. W. and per unit nitrogen.

Accordingly it may be stated that during these early stages there is in the embryo area a marked decrease in protein content per cell, cf. Fig. 10, and possibly also in cell size. My cytological observations are in accord with the latter assumption.

For chick embryo tissues as with other embryonic tissues the general feature is according to LESLIE that RNA per unit of nitrogen decreases during embryogenesis. That the RNA concentration for the early embryo area investigated here instead holds an even level is hardly surprising, considering that the nitrogen concentration actually decreases during the period in question. It is of more interest to establish that there is a steady increase in the RNA concentration during the gastrulation period. STEINERT (1951) has made a similar observation in the early amphibian embryo. Here synthesis of RNA is slight until gastrulation, but the increase becomes considerable as rapidly as true morphogenetic processes set in.

It may be of interest to compare the results from the present investigation with the observations of HERRMANN (1952), who has investigated muscle development in the early chick embryo. HERRMANN's figure for RNA per cell, 5.09×10^{-12} g, referring to somites from embryos of 40 hours' incubation, is in fairly good agreement with the corresponding figure for the whole embryo area, 6.01×10^{-12} g, found in the present investigation. Both values then imply a DNA content per cell of 2.6×10^{-12} g.

Furthermore the same author is also of the opinion that wide variations in the rate of cell multiplication occur in his material. Otherwise such spurts of cell multiplication have been reported by for instance SCHMALHAUSEN (1926) and HAMBURGER (1948, 1952).

In the foregoing the application of the quotient RNA/DNA for indicating periods of cell multiplication and cell differentiation respectively has just been demonstrated. If the same way of regarding the RNA/DNA quotient is applied to the embryo profiles in Fig. 12, we arrive at the conclusion that within the investigated embryo areas Hensen's node and the head region form centres for cell multiplication, while there should be a more decided protein incorporation in the cells of the adjacent parts.

In fact this conception corresponds to the general opinion among embryologists, who have long been aware of a spatial pattern of developmental activity in the chick embryo, cf. SPRATT (1952). Mainly two centres of morphogenetic and histogenetic activity have been designated: Hensen's node and the anterior end of the neural axis. Up to now, however, the observations which might be interpreted as indicating an underlying pattern of physiological and metabolic activity have been lacking from an accurate characterization of such an activity.

If we turn our attention to the free nucleotides, it can be stated that this fraction roughly corresponds to the acid-soluble fraction of the Schmidt-Thannhauser method, obtained by first extracting the tissue in trichloroacetic acid.

Qualitative and quantitative data for these nucleotides will not be given until a later communication.

For the free nucleotides mention has already been made of the striking inverse relation which appears in the present investigation between this fraction and DNA. However this connection must not be stressed too much, as it is obvious that the nucleotide fraction will comprise also material which is not directly linked to DNA metabolism, like DPN etc.

When attempting to explain the variations in DNA concentration in the chick embryo there is one factor which must be seriously taken into consideration: the embryo may have a supply of DNA in its immediate vicinity, which is incorporated during the earliest period of its development. Under such circumstances, wide changes in the accumulation rate of DNA in the embryo cells are likely to occur.

HOFF-JØRGENSEN (1954) asserts that the chick embryo is so well supplied with DNA that there is no discernible synthesis of DNA in the hen's egg until the fourth day of incubation. As his analyses comprise the whole egg (white, yolk and embryo), there is no explanation as to in which part of the egg this reserve is localized.

However in the present investigation it has been possible to demonstrate clearly that embryo cells cultivated in vitro on SPRATT's minimum glucose medium really are capable of DNA synthesis. Therefore if HOFF-JØRGENSEN's data actually refer to DNA alone, the present findings seem to imply that there is a continuous decomposition of DNA in the egg running parallel to synthesis, the former process not being clearly surpassed until the fourth day of incubation.

FRAENKEL-CONRAT *et al.* (1952) have arrived at the result that avidin, which is a component of the white in the hen's egg, actually is a DNA protein. This DNA supply is not very large, in fact there is only 0.02 mg of it in the whole white of an egg, GYÖRGY and ROSE (1942). The occurrence of DNA in the egg white is of great interest especially when considering NEW's (1956) observation that there is remarkable transfer of fluid material through the early blastoderm. Whether there really is a decided uptake of

avidin in the embryo cells in the earliest stages can scarcely be estimated for the present, but it is hoped that further experiments with embryos cultivated in vitro will provide complete information in this respect.

In the present case it is of special importance to obtain complete information as to whether incorporated (and adhering) yolk granules contain any DNA, and if such is the case, whether they are responsible for any variations in the DNA concentration of the embryo cells.

However neither by the usual chemical methods nor by histochemical methods has it been possible for the author to establish definitely the occurrence of DNA in the yolk granules.

MARZA and MARZA (1935) report a weak positive reaction of yolk granules when applying the Feulgen method to yolk material from hen's egg, but they are of the opinion that it cannot be caused by DNA. BRACHET (1950), who has investigated the yolk in the amphibian egg without finding any DNA in it, expresses his conviction of there being no DNA in the yolk of oocytes. Even with knowledge of the findings of, among others HOFF-JØRGENSEN (1954) and SZE (1953), BRACHET (1957) still has a rather critical view as to the nature of such cytoplasmatic DNA.

Finally one cannot exclude the possibility that there actually are variations in DNA content per cell in the early chick embryo. However the hypothesis that the amount of DNA per chromosome set is constant for a given species and that DNA is exclusively found in the nucleus of the cell has proved to be of so general validity that suggestions of a deviation from it must be based on very conclusive proofs.

Nevertheless such proofs have been furnished for early developmental stages of some invertebrates and of the frog, cf. BRACHET (1957). Of special interest in the present case is SZE's (1953) observation that in the frog embryo the content of DNA per cell decreases during embryogenesis from an originally high value to one which agrees in the main with values for liver nuclei from adult animals. The decrease in DNA content is very rapid before gastrulation but relatively slow thereafter, the break in the course being situated somewhere near the onset of gastrulation.

In this connection it may be recalled that gastrulation in the hen's egg takes place during the first day of incubation and the process is reflected in the formation of the primitive streak. Bearing in mind the conditions in the amphibian egg, it would

not be quite surprising to find some departure from the DNA constancy per cell in the avian egg at a corresponding period.

Now it can be said that the figures in Table I offer a direct answer to this question, as they in fact indicate marked differences in the DNA content per cell during embryogenesis. But, as already stated, these calculations are for the present very incomplete, and accordingly we still have to leave the question open as matters stand now.

Summary.

1. In chick embryos incubated for 0—60 hours the amounts of DNA, RNA and nucleotides have been evaluated per units of relative weight (R. W.) and nitrogen respectively. Some information is given about the whole blastoderms, otherwise the analyses refer exclusively to the central "embryo area" of the blastoderms.

2. An estimation of the content per cell has also been attained by using DNA as a basis of reference. However the results from calculations of the DNA-amount per cell indicate some inconstancy for this quantity during development.

3. Analyses of embryo areas cultivated in vitro have definitely proved the existence of an appreciable DNA-synthesis in embryos explanted at the 14—29-hour-stages.

4. The results suggest the existence of a regular alternation between periods of cell multiplication and cell differentiation in the embryo area. Separate analyses of the different parts within this area further indicate that there is an intensified cell multiplication, localized to certain fixed regions.

5. The results are discussed in relation to previous investigations bearing upon the same problems.

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Der Einfluss von Hypoxie auf das Bluthistamin, die Plasma-Protein-Concentration und die Leucocytenzahl des Meerschweinchens.

Von

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Unter O_2-Mangel verändert sich je nach seiner Dauer und Stärke die Zusammensetzung des Blutes. Am bekanntesten ist die secundäre Polycytaemie. Zu den überwiegend flüchtigen Veränderungen gehört der Anstieg der Plasma-Protein-Concentration, hauptsächlich in der ersten Zeit des O_2-Mangels. Ein solcher Anstieg ist mehrfach bei Mensch und Tier beschrieben worden, teilweise in Abhängigkeit vom Grad des Unterdruckes (SUNDSTROEM und MICHAELS 1942, HURTADO, MERINO und DELGADO 1945, ASMUSSEN und NIELSEN 1945). Bei einem langsamen Höhenaufstieg in Schritten scheint sich die Plasma-Protein-Concentration nicht zu verändern (HOUSTONE und RILEY 1947).

Über die Ursache dieser Concentrationsänderung ist nichts Sicheres bekannt. Theoretisch könnte man sich vorstellen, dass unter O_2-Mangelbedingungen Stoffwechselprodukte im Gewebe gebildet werden, die nur langsam vom Ort ihrer Bildung diffundieren, den osmotischen Druck im Gewebe erhöhen und dadurch dem Plasma Wasser entziehen. Der O_2-Mangel könnte aber auch über die Bildung von vasoaktiven Substanzen die Permeabilität der Kapillaren so verändern, dass Wasser aus dem Plasma austritt. Histamin gehört zu solchen vasoaktiven Substanzen, es ist aber

nicht bekannt, ob Histamin mit dieser Erhöhung der Plasma-Protein-Concentration in einem Zusammenhang steht.

Eine beträchtliche Plasma-Histaminzunahme unter akuter Hypoxie (bis zu 3 Std.) is von EICHLER, SPEDA und WOLFF (1943) bei der Katze, die mit einem 10-prozentigen O_2-Mangelgemisch beatmet worden war, beschrieben worden. Beim Menschen haben FABINYI und SZEBEHELYI (1948) ebenfalls mit einem 10-prozentigen O_2-Mangelgemisch eine Histaminsteigerung von ca 40 Prozent beobachtet. Untersuchungen von BURKHARDT *et al.* (195[illegible] b) beim Hund, der Katze und dem Menschen konnten ebensowenig wie PEPE und VACCA (1954) beim Menschen diese Befunde bestätigen.

Unter anhaltender Hypoxie entsprechend 5,500 m Höhe haben BURKHARDT, FLICKINGER und ADLER (1949) beim Hund einen Histaminanstieg auf das 10-fache und mehr mit einem Maximum am 4. Tag gefunden; nach 8 Tagen waren die Ausgangswerte wieder erreicht. BURKHARDT *et al.* (1951 a) haben aber die Befunde später teilweise widerrufen. Bei langen Beobachtungszeiten schwankten nämlich die Werte erheblich und nahmen auch in einigen Fällen progressiv zu.

Unter akuter Hypoxie ist mehrfach ebenfalls eine Leucocytose beschrieben worden (CRESS, CLARE und GELLHORN 1943, HURTADO *et al.* 1945). Dagegen unterscheiden sich die Leucocytenwerte der Bewohner grosser Höhen nicht von den Normalwerten (HURTADO *et al.* 1945).

Absicht der vorliegenden Arbeit ist, die Wirkung von Hypoxie auf das Bluthistamin, die Plasma-Protein-Concentration und die Leucocytenzahl zu studieren. Dabei ist der Einfluss von Antihistamin unter diesen Bedingungen untersucht worden.

Methodik.

350—500 g schwere Meerschweinchen wurden in einer Unterdruckkammer Hypoxie entsprechend einer Höhe von 6,000 m ausgesetzt. Das Material umfasste 100 Tiere, wovon 20 als Kontrollen dienten. Der Unterdruckversuch mit den übrigen Tieren wurde nach verschieden langer Zeit — zwischen 1 Stunde und 3 Wochen — abgebrochen. Jeweils 4—7 Tieren waren in einer Gruppe zusammengefasst.

In drei Einzelfällen wurde als Vergleich ein O_2-Mangelgemisch von 9.8 Prozent O_2 in N_2 an Stelle von Hypoxie durch Unterdruck verwendet.

Den Tieren wurde in Äthernarkose mit einen heparinisierten Spitze durch Punktion der Vena cava inferior Blut abgenommen.

Für die Histaminanalyse wurden 5 ml Vollblut mit 25 ml Aceton gemischt. Nach Umschütteln und Aufbewahren bei +4° C wurde das Gemisch filtriert und Aceton im Vacuum bei Zimmertemperatur abgedunstet. Der Extrakt konnte danach direkt für die biologische Testung verwendet werden, die am isolierten Meerschweinchenileum in Tyrodelösung vorgenommen wurde. Für die Analyse wurde das Ileum gegen 5-Hydroxytryptamin durch Zusätze von Dihydroergotamin (5 μg per ml Badflüssigkeit) 10 Min. lang unempfindlich gemacht. Nach Abschluss der Testserie wurde kontolliert, dass die gesamte stimulierende Wirkung des Extraktes auf den Meerschweinchendarm mit Antihistamin (Lergitin®, Recip) zu blockieren war.

Bei einer Anzahl von Kontrolltieren so wie von hypoxieausgesetzten Tieren war ein Teil des Extraktes ausserdem durch Adsorption an Amberlite IRC-50, wie beschrieben von Dunér und Pernow (1956, und in Druck) gereinigt worden. In einer Versuchsserie von Normal- und Unterdrucktieren wurde das Blut centrifugiert und der Histamingehalt des Plasmas und der Blutkörperchen getrennt bestimmt.

Die erhaltenen Histaminwerte beziehen sich auf die Histaminbase.

Die Plasma-Protein-Concentration[1] wurde nach der Mikrokjeldahlmethode bestimmt.

Um subjektive Fehler möglichst zu vermeiden, war demjenigen, der die Histamin- und Eiweissconcentration bestimmt, unbekannt, welcher Versuchsabschnitt vorlag.

Als Antihistamin wurde Pyribenzamin® (Ciba), in Dosen von 2 mg per kg Körpergewicht alle 12 Std subcutan verabreicht.

Ergebnisse.

1. Das Bluthistamin unter Hypoxie.

Der Histamingehalt des Vollblutes von 20 Kontrolltieren betrug 0.02—0.12 μg per ml, Mittelwert 0.07 ± 0.009 (s. Tab. I). Dieser Wert stimmt mit den Angaben der Literatur überein (Code 1939, Rocha e Silva und Essex 1942). Unter Hypoxie stieg der Histamingehalt und erreichte sein Maximum nach etwa 48 Std. Der höchste Wert war 0.47 μg per ml (Abb. 1). Der Unterschied zwischen dem Histamingehalt der Kontrolltiere und der 2—5 Tage hypoxieausgesetzten Tiere ist hochsignifikant ($P<0.001$). Nach etwa 10 Tagen Hypoxie zeigten die Histaminwerte eine Tendenz abzusinken, und bei den Tieren, die 3 Wochen in Hypoxie waren, lagen fast normale Werte vor. Die Streuung der Histaminwerte der hypoxieausgesetzten Tiere war durchweg sehr gross, vor allem bei den Tieren, die sich länger als 48 Std. im Unterdruck befanden.

[1] Die Plasma-Protein-Analysen wurden im klinisch-chemischen Laboratorium des Karolinska sjukhuset ausgeführt.

Tabelle I.

Histamin im Vollblut, Plasma-Protein und Leucocyten von Kontrolltieren, Unterdrucktieren, antihistamin-behandelten Kontrolltieren und antihistamin-behandelten Unterdrucktieren.

Die Zahlen geben die Werte 2—3 Tage nach Beginn der Behandlung an.

	Histamin im Vollblut μg per ml	Plasma-Protein g per 100 ml	Leucocyten per μg Blut
Kontrolltiere	0.07 ± 0.009 (0.02 — 0.12) n = 20	4.87 ± 0.07 (4.45 — 5.35) n = 17	6,800 ± 644 (4,400 — 9,200) n = 10
Unterdrucktiere	0.25 ± 0.02 (0.14 — 0.47) n = 20	5.53 ± 0.09 (4.50 — 7.07) n = 23	25,500 ± 1,915 (17,800 — 29,300) n = 8
Antihistaminbehandelte Kontrolltiere	0.15 ± 0.02 (0.07 — 0.22) n = 9	4.63 ± 0.12 (4.20 — 5.33) n = 10	5,400 ± 559 (3,000 — 8,300) n = 10
Antihistaminbehandelte Unterdrucktiere	0.35 ± 0.07 (0.18 — 0.52) n = 9	5.06 ± 0.21 (4.52 — 6.91) n = 10	12,000 ± 775 (9,600 — 17,300) n = 10

Tabelle II.

Verteilung von Histamin zwischen Plasma und Blutkörperchen bei Kontrolltieren und Unterdrucktieren mit mässigem und starkem Histaminanstieg.

	Histamin im Plasma μg per ml	Histamin in den Blutkörperchen μg per ml	Totalhistamin μg per ml
Kontrollen n = 6	0.03 ± 0.005 (0.01 — 0.05)	0.07 ± 0.006 (0.05 — 0.10)	0.10 ± 0.008 (0.06 — 0.12)
Unterdrucktiere mit mässigem Histaminanstieg (n = 5)	0.04 ± 0.009 (0.03 — 0.06)	0.11 ± 0.009 (0.09 — 0.12)	0.15 ± 0.009 (0.12 — 0.17)
Unterdrucktiere mit starken Histaminanstieg (n = 6)	0.09 ± 0.002 (0.05 — 0.15)	0.28 ± 0.06 (0.13 — 0.56)	0.37 ± 0.06 (0.15 — 0.66)

Die drei einem O_2-Mangelgemisch 60 Std. lang ausgesetzte Meerschweinchen unterschieden sich in ihren Werten nicht von den Tieren im Unterdruck.

Die Verteilung von Histamin zwischen Plasma und Blutkörperchen ist aus Tab. II zu ersehen. Bei 6 Kontrolltieren mit einem mittleren Histamingehalt von 0.10 μg per ml Blut befanden sich im Durchschnitt 0.03 μg Histamin im Plasma und 0.07 μg in den

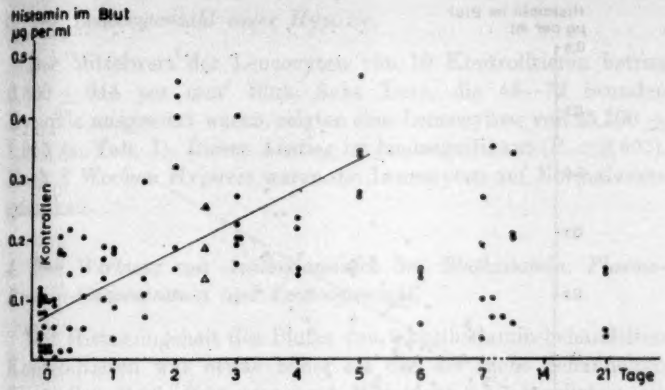

Abb. 1. Der Einfluss von Hypoxie auf den Histamingehalt des Blutes. Regressionslinie für den Histaminanstieg während der ersten 5 Tage, $y = 0.002x + 0.09$. Regressionskoeffizient $r = 0.66$.

• = Tiere in 6,000 m Unterdruck.
△ = Tiere in Hypoxiegas (9.8% O_2 in N_2)

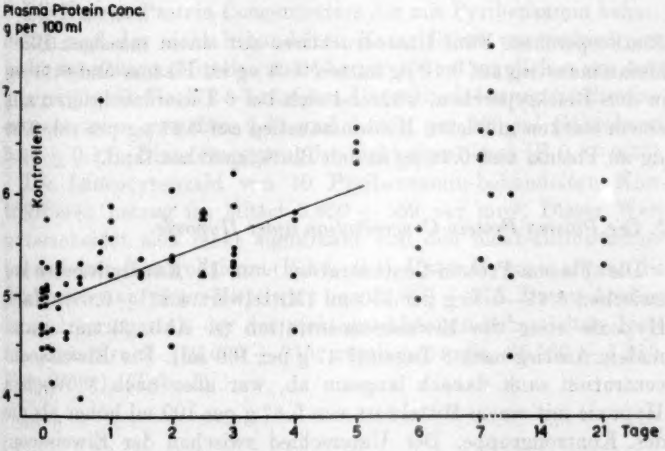

Abb. 2. Der Einfluss von Hypoxie auf die Plasma-Protein-Concentration. Regressionslinie für den Proteinanstieg während der ersten 5 Tage, $y = 0.010x + 4.91$. $r = 0.61$.

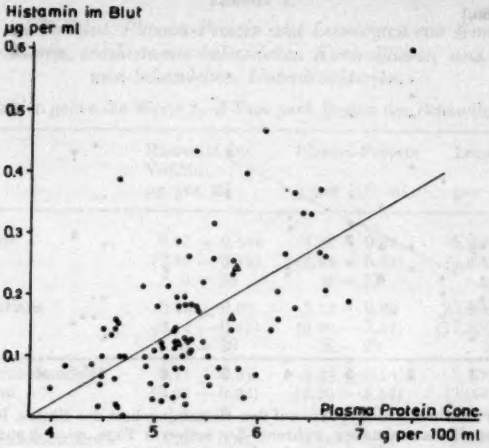

Abb. 3. Die Korrelation zwischen Bluthistamin und Plasma-Protein-Concentration. Gleichung der Regressionslinie: $y = 0.100 \times - 0.38$. $r = 0.59$.

Blutkörperchen. Fünf Unterdrucktiere mit einem mässigen Bluthistaminanstieg auf 0.15 µg hatten 0.04 µg im Plasma und 0.11 µg in den Blutkörperchen, während sich bei 6 Unterdrucktieren mit einem starken mittleren Histaminanstieg auf 0.37 µg per ml, 0.09 µg im Plasma und 0.28 µg in den Blutkörperchen fand.

2. Die Plasma-Protein-Concentration unter Hypoxie.

Die Plasma-Protein-Concentration von 17 Kontrolltieren lag zwischen 4.45—5.35 g per 100 ml (Mittelwert 4.87 ± 0.07). Nach Hypoxie stieg die Eiweissconcentration (s. Abb. 2) mit maximalem Anstieg nach 8 Tagen (7.47 g per 100 ml). Die Eiweissconcentration sank danach langsam ab, war aber nach 3 Wochen Hypoxie mit einem Mittelwert von 5.67 g per 100 ml höher als die der Kontrollgruppe. Der Unterschied zwischen der Eiweissconcentration der Kontrolltiere und der 18 Std. bis 14 Tage hypoxieausgesetzten Tieren war hochsignifikant ($P < 0.001$).

Das Verhalten zwischen Bluthistamin und Plasma-Protein-Concentration ist aus Abb. 3 ersichtlich.

3. Die Leucocytenzahl unter Hypoxie.

Der Mittelwert der Leucocyten von 10 Kontrolltieren betrug 6,800 ± 644 per mm³ Blut. Acht Tiere, die 48—72 Stunden Hypoxie ausgesetzt waren, zeigten eine Leucocytose von 25,500 ± 1,915 (s. Tab. I). Dieser Anstieg ist hochsignifikant ($P < 0.001$). Nach 3 Wochen Hypoxie waren die Leucocyten auf Normalwerte gesunken.

4. Die Wirkung von Antihistaminica auf Bluthistamin, Plasma-Protein-Concentration und Leucocytenzahl.

Der Histamingehalt des Blutes von 9 antihistamin-behandelten Kontrolltieren war etwas höher als der der nicht behandelten Kontrollen (0.07—0.22 μg per ml, Mittelwert 0.15, verglichen mit 0.02—0.12 μg per ml, Mittelwert 0.07). Nach 3 Tagen Hypoxie war der Histamingehalt mit einem Mittelwert von 0.35 μg und einem Höchstwert von 0.52 μg per ml signifikant höher. Er lag jetzt in derselben Grössenordnung wie der von den Tieren, die nicht mit Antihistaminica behandelt, sondern nur Hypoxie ausgesetzt waren.

Die Plasma-Protein-Concentration der mit Pyribenzamin behandelten und der nicht behandelten Kontrolltiere unterschied sich nicht signifikant (Mittelwert 4.63 g per 100 ml verglichen mit 4.87 g per 100 ml). Nach 2—3-tägigem Unterdruck betrug die Eiweisskonzentration zwischen 4.52 und 6.91 g per 100 ml (Mittelwert 5.06 ± 0.21). Diese Steigerung ist nicht signifikant (P 0.1—0.05).

Die Leucocytenzahl von 10 Pyribenzamin-behandelten Kontrolltieren betrug im Mittel 5,400 ± 559 per mm³. Dieser Wert unterscheidet sich nicht signifikant von den nicht-antihistamin-behandelten Kontrolltieren (P 0.2—0.1). Unter Hypoxie stiegen die Leucocyten auf einen Mittelwert von 12,000 ± 775. Dieser Anstieg ist viel geringer als der der nicht-antihistamin-behandelten Tiere im Unterdruck (12,000 ± 775 verglichen mit 25,500 ± 1,915, $P < 0.001$), (Tab. I).

Besprechung der Ergebnisse.

Nach den vorliegenden Untersuchungsergebnissen sind unter Hypoxie die Bluthistaminwerte angestiegen. Die Verteilung von Histamin zwischen Plasma und Blutkörperchen ändert sich dabei

selbst bei sehr starker Vermehrung der Gesamthistaminmenge nicht, wobei ein Verhältnis Histamin im Plasma: Histamin in der Blutkörperchen von etwa 1:3 erhalten bleibt. Der im Zusammenhang mit Muskelarbeit auftretenden Histaminanstieg beim Menschen betrifft in ähnlicher Weise ebenfalls die Plasma- wie die Blutkörperchenfraktion (DUNÉR und PERNOW, in Vorbereitung).

Neben dem Bluthistamin sind auch die Plasma-Protein-Concentration und die Leucocyten unter Hypoxie gestiegen. Die Frage, ob der Anstieg dieser Grössen in einem ursächlichen Zummenhang steht oder ob es sich dabei um ein paralleles Phänomen handelt, ist schwer zu beantworten. Unter Behandlung der Tiere mit Antihistamin bei gleichzeitiger Hypoxie steigt der Histamingehalt des Blutes in demselben Umfang wie bei den nicht-antihistamin-behandelten Tieren. Dies stimmt mit der allgemeinen Auffassung überein, dass Antihistamin die Bildung von Histamin nicht beinflusst, jedoch die Wirkung des Histamines blockieren kann. Es ist daher nicht unmöglich, dass die ausgebliebene Steigerung des Plasma-Protein-Concentration und der verminderte Anstieg der Leucocyten, der bei den antihistamin-behandelten Hypoxietieren zu beobachten ist, Ausdruck eines gehemmten Histamineffektes ist.

Zusammenfassung.

1. Es wurde die Wirkung von Hypoxie auf den Histamingehalt des Blutes, die Plasma-Protein-Concentration und die Zahl der Leucocyten des Meerschweinchens untersucht. Die Tiere waren in einer Unterdruckkammer Hypoxie entsprechend einer Höhe von 6,000 m während eines Zeitraumes von 1 Stunde bis zu 3 Wochen ausgesetzt.

2. Ein statistisch signifikanter Anstieg von Histamin, Plasma-Protein-Concentration und Leucocytenzahl konnte nachgewiesen werden. Die höchsten Werte waren zwischen 2 und 8 Tagen zu beobachten, danach fielen sie wieder ab.

3. Antihistamin-behandelte und gleichzeitig hypoxieausgesetzte Tiere zeigten einen Bluthistaminanstieg von derselben Grössenordnung wie Tiere, die nur in Hypoxie waren. Demgegenüber änderte sich die Plasma-Protein-Concentration dieser Tiere gegenüber den Kontrolltieren nicht signifikant, während die Leucocyten nur mässig und signifikant geringer als die der nicht mit Antihistamin behandelten Unterdrucktiere stiegen.

4. Die Resultate können darauf hinweisen, dass der Anstieg der Plasma-Protein-Concentration und der der Leucocyten unter Hypoxie durch Histamin verursacht worden ist.

Summary.

1. The effect of hypoxia on histamine in blood, plasma protein concentration and leucocytes was studied in the guinea-pig. The animals were placed in a low pressure chamber with a pressure corresponding to 6,000 m of height during periods of from one hour up to three weeks.

2. A significant increase in blood histamine, plasma protein concentration and leucocytes was observed during this treatment. The highest values were found after 2—8 days whereafter a successive decrease was noticed even during hypoxia.

3. In another series of experiments the animals were treated with an antihistaminic (Pyribenzamine®) during the hypoxia. The histamine in the blood then increased to about the same values as in the non-treated hypoxic animals, while the plasma protein concentration did not change. The number of leucocytes also increased but to a much lower degree than in the non-treated animals.

4. As the antihistaminics block the effect of histamine without inhibiting its release into the tissues, the results might indicate that the increase of the plasma protein concentration and the leucocyte count during hypoxia was induced by histamine.

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Resynthesis of Catechol Hormones in the Cat's Adrenal Medulla.

By

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Different opinions have been expressed as to the rate of resynthesis of the adrenal medullary hormones after release by stimulation of the gland. Experiments by HÖKFELT and MCLEAN (1950) seemed to indicate that the resynthesis occurred rapidly in the rabbit. The authors stimulated the left splanchnic nerve for 18 periods of 1 min during 1 hour and compared the rise in blood pressure with the effect of known amounts of adrenaline. The estimated release of 200 μg adrenaline was considerably larger than that normally found in one gland, about 80 μg.

Similarly HOLLAND and SCHÜMANN (1956) estimated the mean release during repeated 50 min splanchnic stimulation in the cat and found a secretion of 584 μg catechol amines per g gland on an average while the decrease in the stimulated gland was only 256 μg/g, the difference representing the synthesis. The amounts released were estimated by matching the effects of splanchnic stimulation on the blood pressure with known amounts of a mixture of adrenaline and noradrenaline.

On the other hand HÖKFELT (1951) has reported that the catechol amine content of rat's and rabbit's adrenal gland, which fell to very low values after large doses of insulin, required about a week to regain the normal content. These results were confirmed and extended by UDENFRIEND et al. (1953) who estimated the recovery

time of the adrenaline after partial depletion with insulin to about one week, with half restoration in about 72 hours. Using smaller doses of insulin WEST (1951) found complete recovery in 8—10 hours.

BUTTERWORTH and MANN (1957 a, b) depleted the cat's adrenal medulla by acetylcholine and found that the total amount of catechol amines in the adrenal venous plasma was equal to or less than the amines lost from the gland, indicating that no resynthesis had taken place. Not even after 17 hours any increase in the amines in the gland was observed. Only after 2—3 days a small rise was noted, and after 6—7 days there was a marked increase. The relative proportions of the amines indicated that noradrenaline was resynthesized at a faster rate than adrenaline. In further experiments, when a recovery period of one month was allowed, normal amounts were found and the relative and absolute noradrenaline increase had returned to normal.

In the present series of experiments the actual amounts of catechol amines released from the left suprarenal gland of the cat under various kinds of stimulation were determined and related to the amounts present in the right gland before stimulation and the left gland after the end of the experiment. In this way it was attempted to find out whether and under which conditions resynthesis in the gland takes place. The stimuli used were infusion and injection of nicotine and other drugs, and electrical stimulation of the left splanchnic nerve.

Methods.

Cats, anesthetized with nembutal 35 mg/kg were used throughout. The right adrenal was immediately dissected out and used as a control. The adrenal venous blood was collected from a polythene tube inserted in the adreno-lumbar vein peripherally of the adrenal gland after tying all branches except those draining the gland. A loop of thread was placed around the vein close to the vena cava in order to allow either a flow into the animal or through the tube. The animal received heparine intravenously to prevent clotting. When occasionally the lumbar vein joined the renal vein this was used for collection of the adrenal venous blood after tying the adreno-lumbar vein peripherally of the gland. The collected blood was replaced by heparinized cat blood or plasma, sometimes diluted with 10 per cent dextrane in Ringer solution.

Immediately after the injection of the drug the collection of blood started and was continued for 5 minutes. As shown by WADA, HIRANO and TIBA (1938) most of the secretion due to stimulation by nicotine is over in 5 min.

Table I.

Loss in catechol amine content of left suprarenal gland of the cat compared with amount secreted during infusion or injection of nicotine. Figures represent total amounts in μg.

Expt. no.	Dose	Control gland		Stimulated gland		Deficit		Secreted		Resynthesized	
		adr.	noradr.	adr.	noradr.	adr.	noradr.	adr.	noradr.	adr.	noradr.
II	*Infusion* 0.04 mg/kg/min (60′)	150	76	88	50	62	26	11	6.5	—	—
III	*Infusion* 0.13 mg/kg/min (107′)	248	92	158	76	90	16	7	7.5	—	—
IV	*11 injections* 0.1 mg/kg	121	55	89	26	32	29	17	16	—	—
V	*12 injections* 0.3 mg/kg	113	92	89	59	24	33	97	56	73	23
VI	*10 injections* 0.15—0.6 mg/kg	133	80	132	67	1	13	6.2	8.7	5.2	—
VII	*12 injections* 0.1—0.8 mg/kg	202	140	109	60	93	80	98	39	5	—
VIII	*13 injections* 0.05—0.8 mg/kg	86	29	54	23	32	6	21	26	—	20

In the nerve stimulation experiments the left splanchnic nerve was prepared and divided below the diaphragm. It was stimulated with shielded platinum electrodes connected with a Grass stimulator. Square waves of 10 msec duration and with a frequency of 20/sec were used. The stimulus strength was chosen so as to give a maximal blood pressure response during a 5 sec test stimulation. In some experiments fluid electrodes were used.

Adrenal venous blood was collected in ice-cooled vessels. In the experiments with splanchnic stimulation blood was collected during consecutive periods up to 30 minutes without the use of an occluding thread loop and immediately centrifuged. The heparine plasma was kept frozen until assayed. The activity of the plasma was estimated biologically on the cat's blood pressure and the chicken rectal caecum and the amounts of adrenaline and noradrenaline calculated according to Euler (1949).

At the end of the experiment the second adrenal was removed. Each gland was extracted in 10 ml 5 per cent trichloracetic acid. After appropriate dilution of the extracts the catechol amine contents were determined by the fluorimetric method of Euler and Floding (1956). Previous tests have shown excellent agreement between the fluorimetric,

Table II.

Secretion of adrenaline and noradrenaline from the left suprarenal gland of the cat during 5 min periods following varying doses of nicotine.

Nicotine mg/kg i. v.	Suprarenal plasma µg/ml		Per cent adrenaline
	adr.	noradr.	
0.1	0.39	0.35	53
0.2	0.99	0.35	74
0.4	3.0	0.0	100
0.8	2.4	1.8	57
0.4	0.99	0.35	74
0.2	0.26	0.18	59
0.1	0.20	0.15	57

colorimetric and biological estimation of adrenaline and noradrenaline in suprarenal extracts.

Results.

Nicotine. Since nicotine is known to act as a very efficient releaser of catechol amines from the adrenal medulla (RAPELA and HOUSSAY 1952) nicotine tartrate was administered in a series of experiments both as infusion and as repeated injections.

In two experiments nicotine was infused intravenously in doses of 0.04 and 0.13 mg/kg/min during 60 and 107 min respectively. As seen in Table I the amount released was very small as compared with the deficit (the difference in catechol amine content between the control and the stimulated gland). Although the larger dose was sufficient to cause an initial rise in blood pressure, the secretory effect was very small. Still larger doses caused a fall in blood pressure and after some time muscular twitching without increasing the secretion.

In four of the five experiments with i. v. injections of nicotine in doses varying between 0.05 and 0.8 mg per kg the amounts secreted were in excess of the deficit between the control and the stimulated gland. Only in one experiment, however, the difference, indicating resynthesis, was considerable, and amounted to 73 µg adrenaline and 23µg noradrenaline or 65 per cent of the original adrenaline content and 25 per cent of the noradrenaline content. In this experiment 11 injections of 0.3 mg/kg were made, followed by 9 injections of 0.6 mg/kg.

Table III.

Loss in catechol amine content of left suprarenal gland of the cat compared with amount secreted during splanchnic stimulation. Figures represent total amounts in μg.

Exp. no.	Control gland		Stimulated gland		Loss (—) or gain (+)		Secreted		Resynthesized	
	adr.	nor-adr.	adr.	nor-adr.	adr.	nor-adr.	adr.	nor-adr.	adr.	nor-adr.
XI	210	109	104	50	—106	—59	217	118	111	59
XII	147	101	181	132	+ 34	+31	38	16	72	47
XIV	124	21	166	42	+ 42	+21	17	37	59	58
XV	156	21	132	29	— 24	+ 8	8.3	22	—	30
XVI	152	29	149	25	— 3	— 4	3.6	17	< 1	13
XVII	166	84	163	84	— 3	0	31	14	28	14
XVIII	134	126	110	92	— 24	—34	76	119	52	85

Considerable variations in the amounts of catechol amines released during a 5 min period after injection of a given dose of nicotine were noted. As a rule the secretion provoked by consecutive injections decreased in spite of the fact that the stores were not depleted to any higher degree.

The relationship between the dose of nicotine injected and the amount of catechol amines released is illustrated in Table II. From the table it appears that the adrenaline secretion bears a certain relationship to the dose of nicotine, while the noradrenaline release suddenly increases with the highest dose used and resumes the low figures with falling nicotine doses.

A nicotine dose of 0.05 mg/kg has still a small but definite effect on the adrenaline secretion whereas the noradrenaline secretion does not seem to be affected by this dose.

A few experiments with injections of acetylcholine, arecoline, and piperidine showed that these drugs, as expected, caused a release of catechol hormones from the suprarenal gland but it did not appear that the results in any way differed from those obtained with nicotine.

Splanchnic nerve stimulation.

In 7 experients the left splanchnic nerve was stimulated electrically and the catechol amine secretion measured. The periods

of stimulation varied from 30—100 min. The results are summarized in Table III.

In 2 experiments (XII and XIV) the amount of catechol amines found in the stimulated gland after the end of the experiment exceeded that observed in the control gland, suggesting a repletion of its stores as a result of stimulation. The deficit was negligible in expt. XVI and XVII and although the secreted amounts were small they probably represented newly formed catechol amines. A definite reduction of the medullary hormone content in the stimulated gland was observed in expt. XI and XVIII in which the amount of secreted catechols was largest. In both these cases a considerable new formation of both hormones took place. No difference in the formation of adrenaline and noradrenaline was observed in the present experiments (more adrenaline than noradrenaline in 3 expts., the reverse in 3 expts. and approximately equal amounts in 1 expt.). The largest new formation amounted to 325 μg adrenaline per g (52 per cent of the original amount (expt. XI) and 745 μg noradrenaline per g (67 per cent of the original amount) (expt. XVIII). In expts. XIV and XV in which the noradrenaline content in the control gland was unusually low, the percentage of synthesized noradrenaline was even higher.

Discussion.

The present results have added further data to the experiments by HÖKFELT and MCLEAN (1950) and by HOLLAND and SCHÜMANN (1956) which indicated that the release of catechol hormones caused by splanchnic stimulation in the rabbit and in the cat is followed by a rapid resynthesis of the hormones. These results were seemingly at variance with the findings that the catechol depletion caused by insulin is very slowly repaired, or that depletion after acetylcholine likewise takes long time to return to normal. It seems therefore likely that the chromaffin cells have the intrinsic property of being able to replete their stores rapidly, which is certainly the case with the adrenergic nerves.

The slow recovery of the medullary hormones after certain drugs might therefore be interpreted as due to some alteration in the cellular metabolism.

The rapid fall in the secretion of the catechol amines after an injection of nicotine, previously reported by WADA et al. (1938).

may be related to a persistent depolarization as described for ganglionic transmission by ECCLES (1956). An effect of this kind may also be reponsible for the lack of demonstrable secretory action of an infusion of nicotine.

In contrast to the slow turnover of catechol hormones indicated by the experiments of UDENFRIEND et al. (1953) are the findings of a relatively rapid turnover in phaeochrome cell tumours (SJOERDSMA, LEEPER and UDENFRIEND 1957) which also emerges from the observations on hormone production and content in tumours in cases of phaeochromocytoma (EULER 1958). It should also be mentioned that UDENFRIEND and WYNGAARDEN (1956) consider the possibility of a more rapid turnover in the insulin experiments if it is assumed that only a small proportion of the secretory cells are engaged in the release.

The findings of BUTTERWORTH and MANN (1957 a, b) are interesting also from the point of view that acetylcholine is the normal transmitter of the secretory stimulus in nerve stimulation (FELDBERG and MINZ 1932).

From Table II it can be seen that the relative proportion of adrenaline released from the adrenal medulla varies with the dose of nicotine. Increase of the dose from 0.1 to 0.4 mg per kg causes an increased output of adrenaline in the adrenal venous blood while the noradrenaline output is unchanged or diminishes. With a still larger dose, 0.8 mg, both the adrenaline and noradrenaline release is greatly enhanced. It would therefore seem as if the release of the two hormones follows a different pattern. In view of the fact that the two hormones are present in different kinds of cells (HILLARP and HÖKFELT 1953) it appears that the excitation or release mechanism does not go parallel.

Summary.

1. Repeated injections of nicotine tartrate in doses of 0.05—0.8 mg per kg in the cat cause a release of catechol amines in the adrenal venous blood, sometimes exceeding the loss in the gland compared with the control gland.

2. The rapid resynthesis observed in some cases after nicotine injections was not observed when nicotine was infused over a period of 60—107 min in doses of 0.04—0.13 mg/kg/min.

3. Increasing doses of nicotine within the dose range 0.1—0.4

mg/kg increased the release of adrenaline while doses of 0.8 mg/kg caused a rise also of noradrenaline.

4. The release of catechol amines during electrical stimulation of the splanchnic nerve was often considerably in excess of the loss in the stimulated gland. The maximal resynthesis of adrenaline corresponded to 325 μg per g gland and of noradrenaline 745 μg per g gland.

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